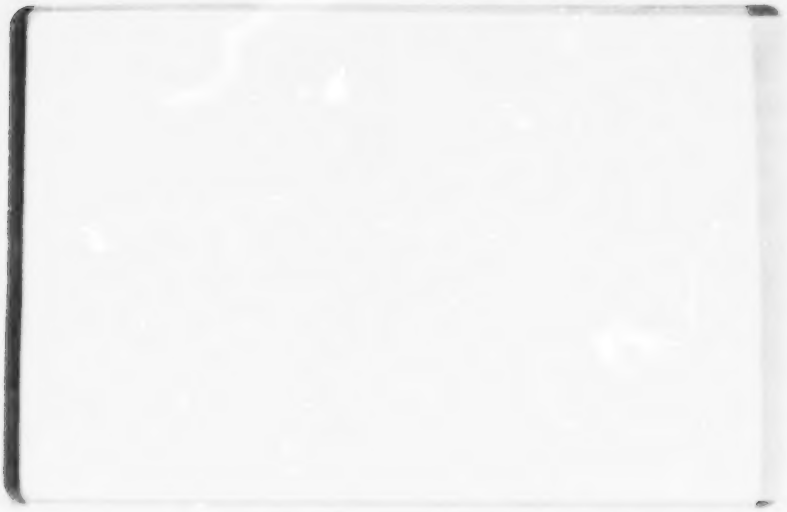

Gemini
BIOCHEMICAL
RESEARCH Ltd

BIOLOGICAL CONTROL OF METHANE IN COAL MINES, COAL STORAGE SILOS AND SHIP HOLDS

PHASE IV

A REPORT BY

GEMINI BIOCHEMICAL RESEARCH LTD.

Work on this project was conducted under the auspices of the Canada Centre for Mineral and Energy Technology, Energy Mines and Resources, Canada.

Financial support was provided by CANMET, Canadian Coast Guard (Ship Safety Division) and Cape Breton Development Corporation.

February 1987

D.S.S. File Number	15SQ.23440-6-9030
D.S.S. Contract Serial Number	23440-6-9030/01-SQ
CANMET Project Number	
CANMET Scientific Authority	Dr. R.N. Chakravorty

MICROMEDIA

64 pp

SUMMARY

The small scale experiments used in the previous study were successfully scaled up to allow treatment of 3 tonnes of coal. Two successful tests were carried out using CBDC coal. One test with Sparwood coal gave inconclusive results.

The tests with the CBDC coal showed that the addition of small amounts of a methane oxidizing bacterial culture to the coal resulted in significant lowering of methane levels both in the air space in the silo and in the coal pile when the silo was ventilated. When the silo was sealed oxygen dropped immediately to below detectable levels within a two to three day period.

The poor results obtained when using the Sparwood coal are probably related to the very fine and dry nature of this particular sample of coal.

A simple and inexpensive bioreactor was designed and constructed to produce the methane oxidizing bacteria. The growth kinetics in this bioreactor were superior to those observed in a small stirred tank fermentor. This bioreactor was operated in a fed-batch mode for a period of six months without any deleterious effects on the production of the methane oxidizing bacteria.

A comparison between "sticky" and "non-sticky" cells showed that their biomass and specific activities were quite similar. Differences noted in the previous study are believed to be due to the small scale of the tests used. "Sticky" cells would still be preferred for use with immobilized cells.

The bacteria were stable, retaining over 90% of their activity when stored at room temperature or 4°C. Concentration of the cells caused no loss in activity.

ABRÉGÉ

Les expériences à petite échelle de l'étude préalable ont été répétées à plus grande échelle pour permettre un traitement allant jusqu'à 3 tonnes de charbon. Deux essais, en utilisant le charbon CBDC ont eu d'excellents résultats, tandis qu'un autre essai , en utilisant le charbon Sparwood, n'a donné que des résultats peu convaincants.

Les essais avec le charbon CBDC ont démontré qu'en ajoutant au charbon de petites quantités d'une culture bactériologique oxydant le méthane, l'on peut observer une diminution appréciable des concentrations de méthane dans le volume d'air du silo et dans le tas de charbon lorsque le silo est aéré. Quand le silo est fermé hermétiquement, la teneur en oxygène tombe en dessous d'un seuil détectable sur une période de deux à trois jours.

Les résultats médiocres que l'on a obtenus en utilisant le charbon Sparwood, sont probablement dûs à la nature même de ce charbon dont la texture est très fine et sèche.

Un bioréacteur simple et bon marché a été conçu et assemblé pour produire la bactérie oxydant le méthane. La vitesse de reproduction dans le bioréacteur est supérieur à celle que l'on a observée dans une petite cuve de fermentation sous agitation. Ce bioréacteur a fonctionné en semi-continu pendant une période de six mois sans rencontrer aucun problème pouvant nuire à la production de la bactérie oxydante du méthane.

Une comparaison entre les cellules qui s'agglutinent et celles qui ne s'agglutinent pas a démontré que leur biomasse et leur activité spécifique sont assez semblables. On pense que les différences remarquées pendant l'étude précédente sont dues à la petite envergure des essais réalisés. L'emploi de cellules agglutinantes avec celui de cellules immobiles est toutefois préférable.

La bactérie est stable et garde plus de 90 % de son activité lorsqu'elle est conservée à la température ambiante , ou à 4o C. La concentration des cellules ne réduit pas leur activité.

TABLE OF CONTENTS

Introduction	1
Experimental	5
Results	17
Discussion	44
Conclusions	50
Recommendations for Further Research	52
References	53
Appendix A	54

FIGURES

Figure 1.	Diagramatic Representation of the Bioreactor	7
Figure 2.	Diagramatic Representation of the Silo	11
Figure 3.	Bacterial Activity in the Bioreactor	19
Figure 4.	Bacterial Activity in the Bioreactor	20
Figure 5.	Methane and Oxygen levels during the First Test with CBDC Coal	21
Figure 6.	Carbon Dioxide Levels during the First Test with CBDC Coal	22
Figure 7.	Gas Level Profile during the First Test with CBDC Coal	23
Figure 8.	Gas Levels in the Coal Pile During the Second Test with CBDC Coal	25
Figure 9.	Gas Levels in the Air Space during the Second Test with CBDC Coal	26
Figure 10.	Methane levels During the Second Test with CBDC Coal	27
Figure 11.	Oxygen Levels During the Second Test with CBDC Coal	28
Figure 12.	Carbon Dioxide Levels During the Second Test with CBDC Coal	29
Figure 13.	Gas Level Profile During the Second Test with CBDC Coal	30
Figure 14.	Gas level Profile During the Second Test with CBDC Coal	31
Figure 15.	Methane Emission Rates	33
Figure 16.	Methane Levels During Tests with Sparwood Coal	34

Figure 17.	Oxygen Levels During Tests with Sparwood Coal	35
Figure 18.	Carbon Dioxide Levels During Tests with Sparwood Coal	36
Figure 19.	Comparison of "Sticky" and "Non-sticky" Cells	38
Figure 20.	Effects of Reduced Nitrogen Levels in Medium	39
Figure 21.	Effects of Concentrating the Cells	39
Figure 22.	Stability of the Bacterial Oxidation Activity	41
Figure 23.	Bacterial Oxidation Activity of Immobilized Cells	42

TABLES

Table 1. Description of Coals Used in Study 6
Table 2. Economic Assessment 42

PLATES

Plate 1.	Bioreactor Used in the Study	8
Plate 2.	Model Silo used in the Study	12

ACKNOWLEDGEMENTS

Gemini Biochemical Research Ltd. wishes to acknowledge the support and assistance of CANMET, Transport Canada (Ship Safety) and Cape Breton Development Corporation during the course of this study.

The kind assistance of all mining company personnel is gratefully acknowledged.

Finally, we would like to thank Dr. G. A. Din, Department of Biology, University of Calgary, and Mr. G. Yuzwa, H2O Engineering Ltd., for many helpful discussions and advice.

INTRODUCTION

For the past four years, Gemini Biochemical Research Ltd. has been conducting a research project on the biological control of methane in coal mines and coal storage and transportation vessels. This project is jointly supported by CANMET, Transport Canada (Ship Safety Division) and the Cape Breton Development Corporation. The results of Phase I included a literature search on the properties of methane oxidizing bacteria to identify if conditions in coal mines and coal storage vessels would be suitable environments for these organisms. In addition, a review of the latest research from Russia, Eastern Europe and India was included (1).

The literature review showed that methane oxidizing bacteria had physiological properties which are suitable for their use in coal mining environments. In particular, their pH profiles, temperature optima and nutritional requirements were found to be appropriate.

The survey of the recent field tests in various countries showed that most of the tests yielded positive results when used under actual mining conditions but that there was a paucity of information on the microbiological characteristics of the organisms used and that not enough data were presented to critically assess the validity of the results.

Based on the findings presented in the report from Phase I (1), Phase II was initiated and the results of this phase of the project are summarized below (2).

Water and coal samples were obtained from five mines in Western Canada and two from Cape Breton. Chemical analysis of the water samples and coal leachates from the Western Canadian mines showed that conditions in these mines were

appropriate for the growth of methane oxidizing bacteria. In particular, the pH of the mine waters was in the correct range (pH 7.0-8.5) and there did not appear to be any toxic compounds present in the water. The pH levels in water samples obtained from the Cape Breton mines were found to be higher than expected, most samples being in the range 7.0-8.5. A few samples were acidic but the majority were closer to 8.0. The samples with low pH probably reflect the activities of sulphur oxidizing bacteria on the pyritic sulphur in the coal.

Methane oxidizing bacteria were found to be present in all water samples examined from Western Canadian mines.

One strain of methane oxidizing organism was grown in larger quantities and its growth requirements were partially characterized. The organism grew in a simple inorganic (Bushnell Haas) medium when incubated in a methane/air (20/80; v/v) mixture. The optimal pH (8.0) of this organism was very close to the average pH levels found in the Western Canadian mine waters.

The organisms retained 60% of their initial activity after a period of 100 days in contact with a number of low sulphur Western Canadian coal samples. However, they rapidly lost activity when in contact with an unwashed, high sulphur Cape Breton coal. This loss in activity is believed to be due to the oxidation of pyritic sulphur, present in the coal, to sulphuric acid thus lowering the pH of the liquid phase to below that at which the methane oxidizing bacteria retain maximum activity.

When the methane oxidizing bacteria were added to partially degassed Western Canadian coals, the organisms oxidized the methane as it desorbed from the coal. No methane was detectable after seven days whereas tests with no added bacteria showed 7% methane concentration.

Experiments with a dynamic test set up showed that the organisms were able to reduce the levels of methane in air flowing through a coal bed by as much as 75% (2).

The results obtained during this preliminary experimental phase were sufficiently encouraging that further experiments were conducted. The results from Phase III (3) showed that the small scale experiments carried out during Phase II could be scaled up to allow treatment of 10 Kg quantities of coal. The rates of methane oxidation in the larger model silos were as high as those observed in the small scale tests. No differences in gas composition were noted if gas samples were withdrawn from either the airspace above the coal or from within the coal pile. This showed that diffusion of methane or oxygen was not limiting the rate of methane oxidation for this size of silo.

The pH activity profile of the strain of organisms used was broadened from that used in the previous study. Significant activity was found at lower pH values, particularily in the range 5.0-6.5. The organisms were most active in the range 6.0-8.0.

The strains of organisms isolated in the previous study were more fully characterized, particularily their temperature and pH profiles. Optimum temperature for bacterial activity was shown to be 33°C. Activity dropped rapidly when the temperature was raised above the optimum but only dropped slowly when it was lowered. The organisms were totally inactive at temperatures below -20 and above 40°C but recovered when the temperature was adjusted to more favourable values.

Methods were adopted to allow the organisms to be used with high sulphur Cape Breton coals. Specifically, three methods were found to allow the organisms to be used with the Cape

Breton coals. Firstly, the pH profile of the organisms was adapted to lower vales. Secondly, methods were found to immobilize the organisms so that they did not come in contact with the coal. Thirdly, if the inorganic sulphur was removed the resulting coal did not inactivate the organisms.

Since the results from this project continued to show that a biological method for control of methane was a viable alternative to conventional techniques it was decided to proceed with a further set of experiments. The objectives of Phase IV of the project are outlined below:

(i) To design and construct a model silo capable of holding 2-3 tonnes of coal. At least three tests of the bacterial oxidation method will be carried out using this silo. Two tests will be on Cape Breton coal and one test with a Western Canadian coal.

(ii) To design and construct a large scale bioreactor capable of producing sufficient bacterial cells for the tests. Optimum conditions for the operation of the bioreactor will be studied.

(iii) To scale up the bioscrubber experiments carried out in the previous phase of the project.

(iv) To study the differences between "sticky" and "non-sticky" cells.

EXPERIMENTAL

1 Coals Used in this Study

Two coals were used in this study, a coal from CBDC's wash plant and a washed coal from Westar's Balmer mine (referred to as "Sparwood coal" in the text). The proximate analyses for the coals used are shown in Table 1.

The CBDC coal used in the first test was mined on June 23, 1986. The CBDC coal used in the second test was mined on October 29, 1986. No exact date can be given for the Sparwood coal but it was thought that it would have been mined in the period January 10-15, 1987.

2 Growth of Methane Oxidizing Bacteria

The strain of organism used in this phase of the work was the culture which had been adapted to grow in the small one litre fermentor described in the previous report (3). This organism had been cultured in a fed-batch mode for a period of six months. This strain was further adapted to grow in the bioreactor shown in Figure 1 and described below (Section 3).

The medium in which the organisms were cultured was Bushnell Haas medium consisting of the following ingredients:

Magnesium sulphate	0.2 g
Calcium chloride	0.02 g
Monopotassium phosphate	1.0 g
Dipotassium phosphate	1.0 g
Ammonium nitrate	1.0 g
Ferric chloride	0.05 g
Tap water	1000 ml

The bioreactor was operated in a fed-batch mode by replacing

TABLE 1

COALS USED IN THIS STUDY

	CBDC Washplant	Sparwood
Rank	High volatile bituminous	low volatile bituminous
Sulphur content	1.4%	0.3%
Water content	6.5%	7.5%
Size	+ 1 inch 4.7% +3/4 inch 4.7 +1/2 inch 11.6 +1/4 inch 24.5 +1/8 inch 18.5 +24 mesh 30.0 -24 mesh 6.0	Mostly 100 mesh

80% of the growth medium with sterile fresh medium twice weekly. Air was sparged through the system at a rate of approximately 40 litres per minute. Methane was bled into the air supply at a sufficient rate to give a concentration of between 1 and 2% in the exit gas. The pH and temperature of the medium were not controlled during the operation of the bioreactor. Ambient temperature was approximately 20°C.

3 Description of the Bioreactor

The bioreactors (two were constructed and used in this study) were made from ABS plastic tubing. The main compartment in the bioreactor was 10 cm in diameter and was 2 m high. An external draft tube of 5 cm diamater was connected via 90° elbows and two "T" junctions was fitted as shown in Figure 1. An additional piece of 12 cm tubing was attached above the top junction to prevent overflowing of the reactor when air was sparged through it. The total volume of the reactor was 30 litres but the nominal working volume was reduced to 25 litres due to gas holdup. Air was admitted to the reactor by a piece of tygon tubing which entered through the reactor top and was carried down inside the main compartment to a point approximately 15 cm above the bottom junction. A porous gas sparger was attached to the bottom of this tubing. A second tube exited from just inside the top of the reactor and was vented to the outside of the building for reasons of safety. Methane was bled into the air supply line and the flow rate adjusted so that methane concentrations in the exiting gas were kept within the range 1-2%(v/v).

It should be noted that this was not a sterilizable system but with proper care the bioreactor could be run in a statisfactory manner as discussed in the Results section.

Plate 1 shows the bioreactors in operation.

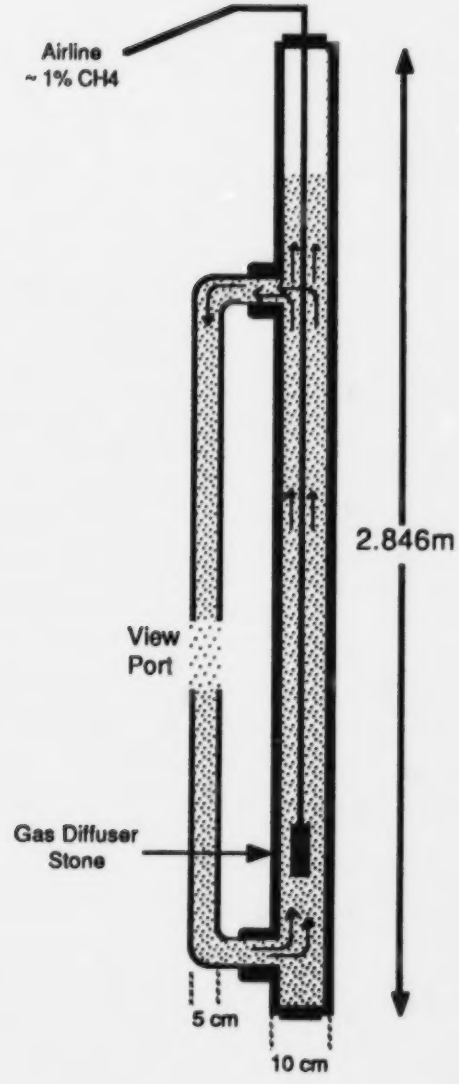

Figure 1.
Diagramatic Representation of the Bioreactor

PLATE 1

BIOREACTOR USED IN THE STUDY

Growth of the organisms was monitored daily by measuring the following parameters:

(i) methane oxidation activity (see Section 9)

(ii) optical density of the culture at 600 nm

(iii) the dry weight of bacterial cells.

In addition, the pH of the culture was measured before each transfer.

4 Model Silo Experiments

A model silo made from steel sheeting was built by a local manufacturer. The silo was 3.3 m high and 1.3 m in diameter and was designed to hold between 2 and 3 tonnes of coal. Gas sampling ports were fitted at 30 cm intervals in the side of the silo as shown in Figure 2. The top and bottom could be sealed with silicone sealant to prevent leakage of air into the silo during experimental runs.

Plate 2 shows the silo in operation.

During an experimental run the silo was filled with the coal being tested, the silo was sealed and air samples were removed and analyzed for oxygen, methane and carbon dioxide. The gases were analyzed daily for approximately two weeks, at which point air was passed across the top of the coal pile by means of a small air pump (air flow rate was approximately 24 litres per minute). This was done to represent the natural ventilation which would occur on an actual coal carrying vessel. Measurement of the gases was continued for another two weeks. The data provided during this phase of the experiment gave an indication of the rate of accumulation of methane and the rate of utilization of oxygen in the absence of bacteria.

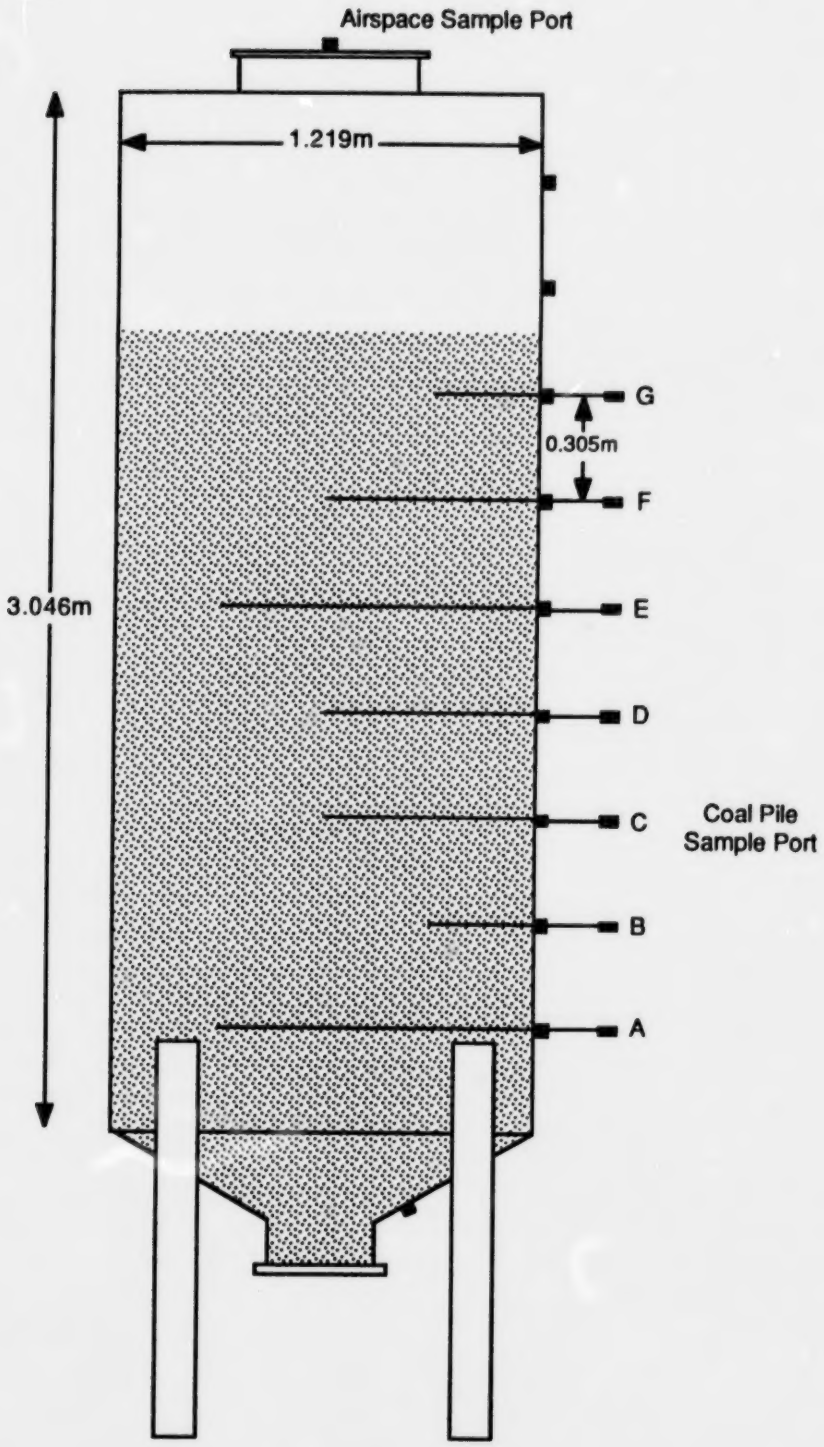

Figure 2.
Diagramatic Representation of the Silo

PLATE 2

MODEL SILO USED IN THE STUDY

The coal was removed, mixed with bacterial culture (2.7%; v/w) and added back to the silo. The bacterial cells were mixed with the coal in 45 gallon drums. No attempt was made to get intimate mixing of the cells and the coal. The amount of bacterial culture added was based on the optimal concentration obtained from experiments performed during the previous study (3). Gases were analyzed for a period of several weeks as described above for the experiment without added bacteria. The cycle of complete sealing followed by a period of forced ventilation was repeated.

When the Sparwood coal was tested a slightly different procedure was adopted. Since this coal was extremely fine and dry it would have been impractical to empty the coal out of the silo, mix it with bacterial culture and replace it (it was feared that the dust created by following this procedure would have a negative effect on the laboratory environment). Instead, the bacterial culture was poured onto the top of the coal in the silo.

5 Differences Between "Sticky" and "Non-sticky" Cells

The "non-sticky" cells were produced as described above. "Sticky" cells were obtained from fresh isolates of cells grown on Bushnell Haas agar plates. Cells from the plates were transferred into 500 ml shake flasks containing 100 mls of liquid Bushnell Haas medium. These flasks were fitted with serum stoppers and rubber membranes as described previously (3). The atmosphere in the flasks was adjusted to 20% with methane and the flasks were incubated at 22°C on a rotatory shaker (300 rpm). The atmosphere was replaced with a fresh methane air mixture twice weekly. The cells were transferred into fresh medium every two weeks.

6 Growth on Media Containing Varying Levels of Nitrogen

In order to assess the effects of varying nitrogen levels on the potential for producing "sticky" cells an experiment was set up to investigate this relationship. A basal salts medium (Bushnell Haas minus the nitrogen components) was made up. Ammonium nitrate was added to this medium at the levels of 10, 20, 50, and 75% of the levels found in Bushnell Haas medium. Aliquots of a "non-sticky" cell suspension from the bio-reactor was added to each flask as described above for producing "sticky" cells. The flasks were incubated in a methane air atmosphere for varying time periods. After incubation the cells were removed, assayed for methane oxidation rates, dried and weighed.

7 Effects of Concentrating the Methane Oxidizing Bacteria

Ten ml aliquots of fermentor grown methane oxidizing bacteria were centrifuged at 10,000 x g for 10 minutes. The supernatants were removed and the pellets were re-suspended in 2, 4, 6, 8 and 10 ml of fresh Bushnell Haas medium. The resuspended cells were added to 100 g Sparwood coal contained in 500 ml shakeflasks fitted with sidearms as described above. Methane was added to the flasks to give final concentrations of approximately 12%. After 24 hours of incubation at 22°C the residual methane concentrations were measured.

8 Stability of the Methane Oxidizing Bacteria

The stability of the methane oxidizing bacteria was measured by removing an aliquot of culture broth from a bioreactor run. The methane oxidizing activity of the aliquot was measured immediately after removal and at daily intervals thereafter. The sample of culture broth was kept at room temperature during this experiment. A comparison of the methane oxidizing activity over the time course of the

experiment gave an indication of the stability of the methane oxidation activity.

9 Standard Method for Measuring Methane Oxidation Rates

During the course of this phase of the study a standardized method for the measurement of the methane oxidizing activity of a culture broth was developed. Six mls of a cell suspension or culture broth were added to a 20 ml serum vial. The vial was capped with a serum cap and 6 mls of methane introduced into the vial via a syringe. The initial methane concentration was measured. The vial was incubated for 5 hours on a rotatory shaker (300 rpm) at 22°C at which point the residual methane was determined. The difference between the initial and final concentrations of methane was a direct measurement of the methane oxidizing activity of the sample. The five hour oxidation rate appeared to be relatively insensitive to the initial methane concentration, in the range 7-15%, thus making the assay useful in correlating methane oxidation rates from different experiments.

10 Economic Assessment

The future commercial potential of this biological process depends on the costs to treat the coal. In order to arrive at an estimate for these costs it is necessary to calculate a number of variables for the process. In order to calculate the cost to treat one tonne of coal it will be necessary to determine the amount of culture broth which will be required to be added to the coal to oxidize the methane desorbing from it. The cost of producing this quantity of cells will also have to be determined.

Results from the previous phase of the study (3) showed that the amount of bacterial culture which was required for efficient methane oxidation rates was in the range 2-5%

(v/w). This is equivalent to approximately 20 litres per tonne.

The second parameter was calculated in a similar manner as in the previous study except that data were obtained from the larger scale bioreactors. The data obtained from the 30 litre bioreactors were scaled up to arrive at an approximate cost for treating 1000 and 5000 tons of coal per day.

11 Analytical Techniques

Necessary chemical and proximate analyses of the coals used in this project were carried out by a commercial laboratory following established ASTM methods or were provided by the coal companies.

Gases were analyzed by gas chromatography using inhouse facilities. The experimental details of this technique and other methods are outlined in Appendix A.

RESULTS

1 Organisms

The strain of organism used in this study was the strain which had been adapted for growth in the one litre fermentor as previously described. This strain grew readily in the fermentor and reached maximum methane oxidizing activity in a period of 4-5 days. This strain is identical to the strain called "non sticky". It grew as a very faintly pigmented culture which was evenly dispersed in the culture broth. There was no flocculation or settling when the culture was allowed to stand undisturbed.

This culture was further adapted to grow in the 30 litre bioreactors. Growth in these bioreactors was very similar to growth in the fermentor except that maximum activity was reached a day or two earlier i.e 2-4 days rather than 4-5 days. See next section for more details on the growth in the bioreactors.

In addition to this "non-sticky" culture, "sticky" cells were isolated freshly from agar plates and were grown in small shake flasks. These cultures had more pigment than the "non-sticky" cells and were much more granular in appearance and tended to settle out of suspension on standing.

2 Growth in the Bioreactor

The bioreactor shown in Figure 1 was assembled and tested for the growth of the "non-sticky" methane oxidizing bacteria described previously (3). The bioreactor could not be sterlized by thermal means due to the nature of the construction materials therefore before the first run it was rinsed with a weak formaldehyde solution (5%) to kill off any contaminating organisms. The medium was prepared by

autoclaving a concentrated solution of the inorganic salts followed by dilution with tap water which had been filter sterilized.

The bioreactor was inoculated with 2 litres of fermentor grown "non-sticky" cells. The bioreactor was operated non-stop in a fed-batch mode for a period of 5 months. Twice a week 80% of the culture fluid was removed and was replaced with fresh, filter sterilized medium.

After a preliminary start up period, the bioreactor could be run in a reproducible manner. Figures 3 and 4 show the growth kinetics for a number of runs. As can be seen from the Figures, maximum methane oxidizing activity was reached in a period of 3-4 days. For the majority of these runs the specific activity (methane oxidizing activity per unit of cell biomass) was fairly constant. This would indicate that the culture is stable and that little contamination by non-methane oxidizing bacteria is ocurring since this would greatly reduce the specific activity.

3 Model Silo Experiments

Three runs were carried out using the model silo, two with CBDC washplant coal and one with Sparwood coal. The data obtained from the first run with CBDC coal are shown in Figures 5, 6 and 7.

With the silo closed and no added bacteria there is a rapid rise in methane concentration accompanied by a decrease in oxygen concentration. No carbon dioxide was detected. The decrease in oxygen was due to the addition of methane rather than consumption of oxygen since the ratio of nitrogen to oxygen was constant during this part of the experiment. After a period of one week the silo was opened to allow access of fresh air and to allow some of the methane to escape. As can be seen from Figure 5, the levels of methane

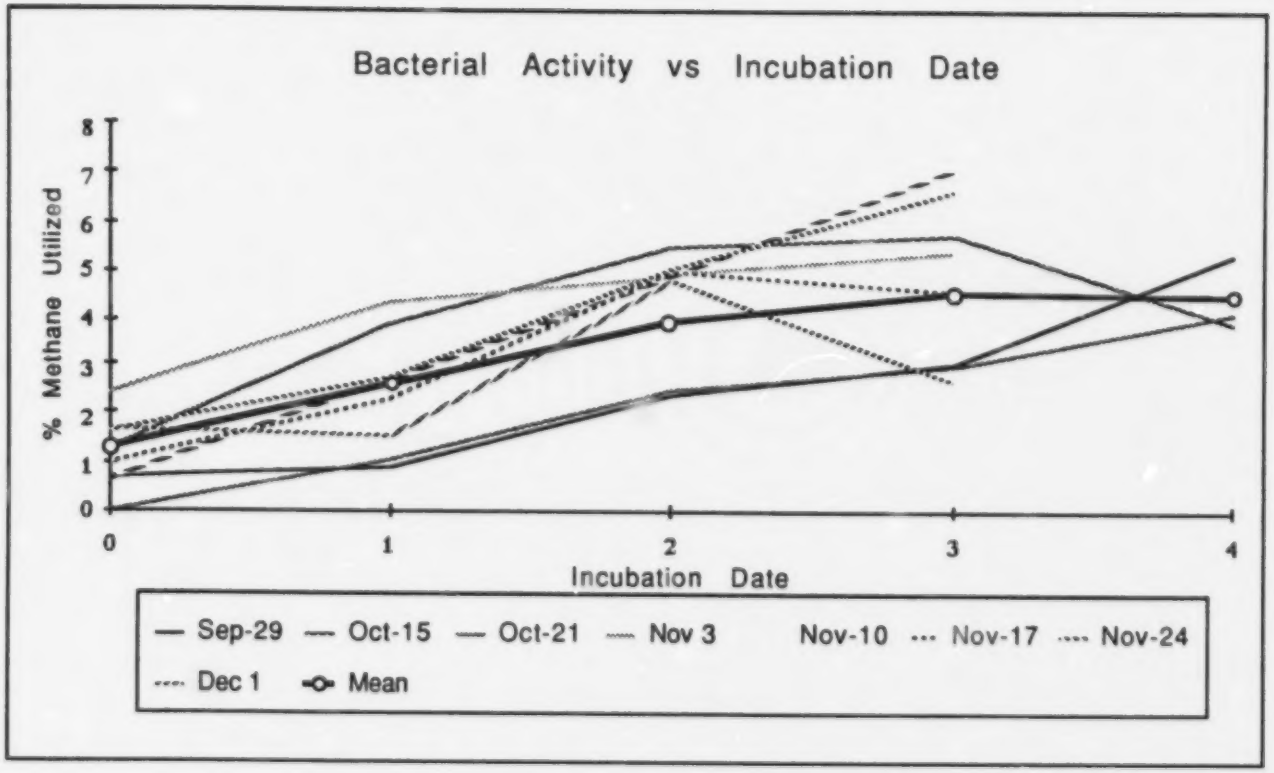

Figure 3.
Bacterial Activity in the Bioreactor

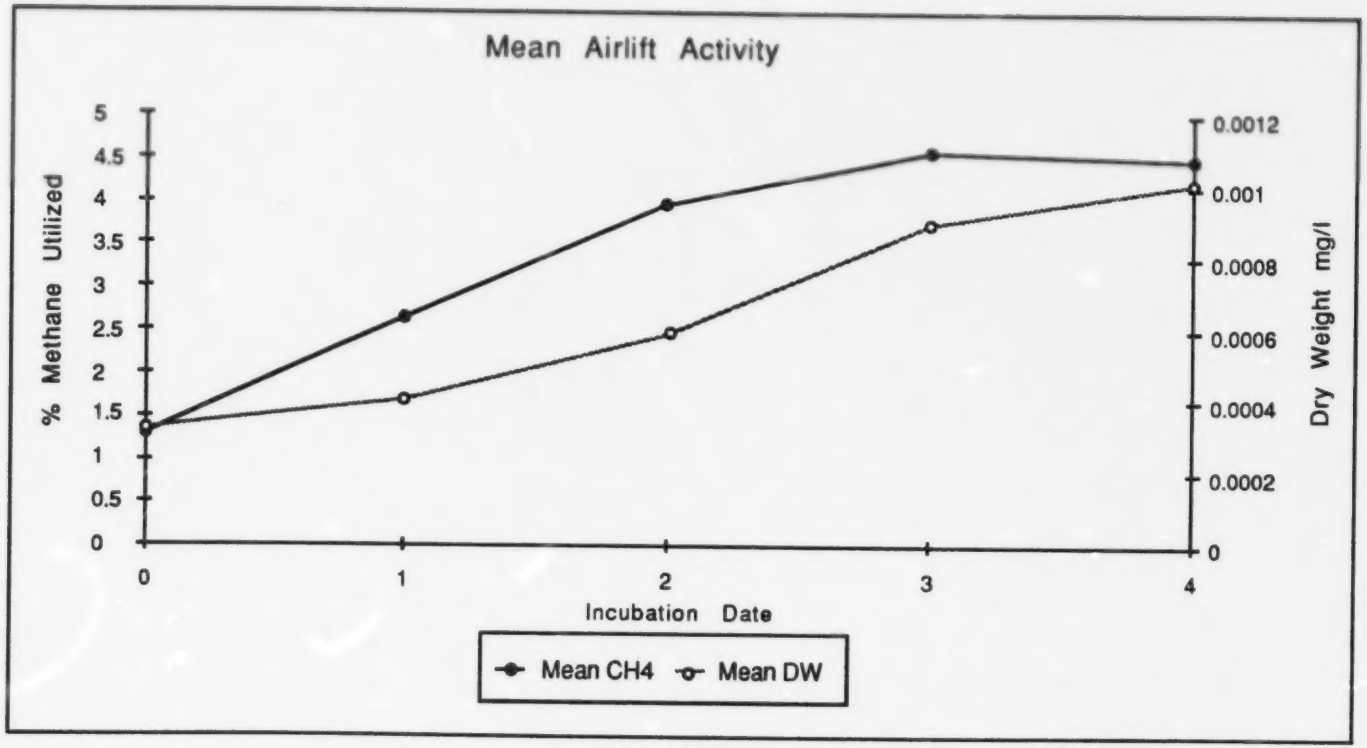

Figure 4.
Bacterial Activity in the Bioreactor

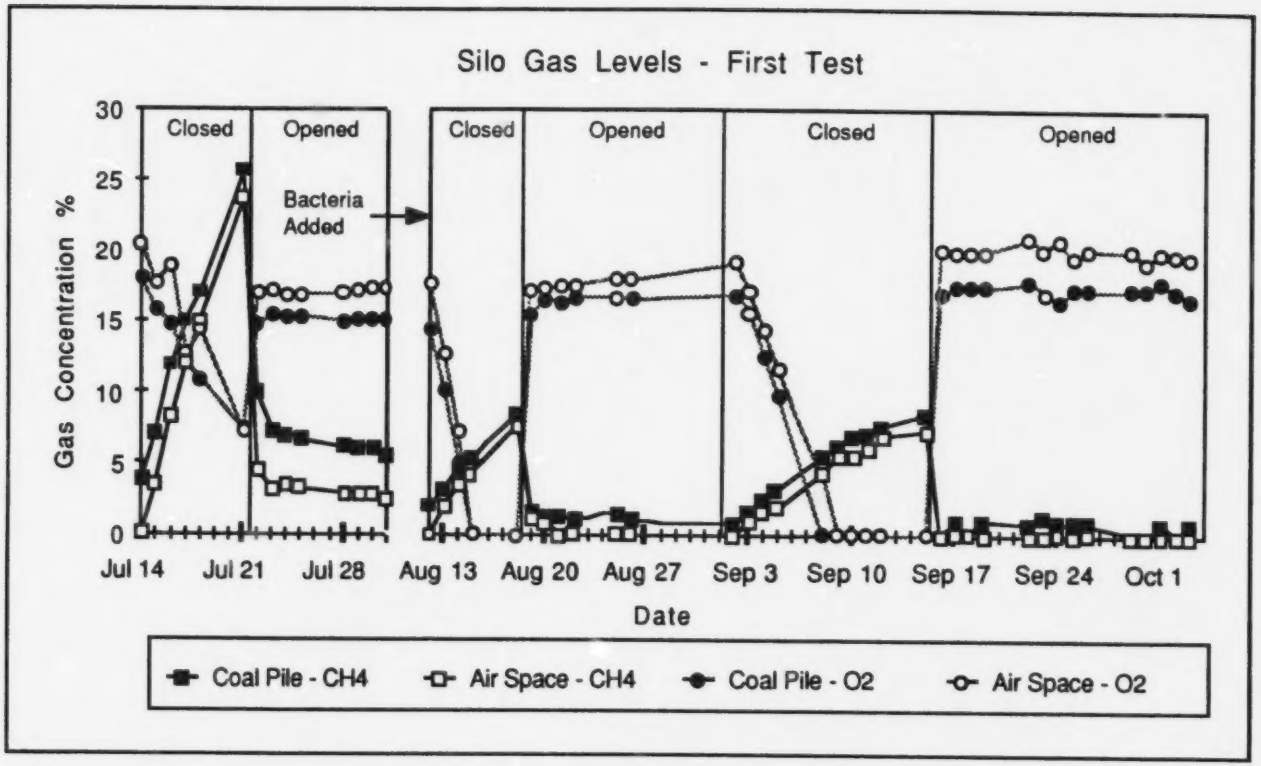

Figure 5.
Methane and Oxygen Levels During the First Test with CBDC Coal

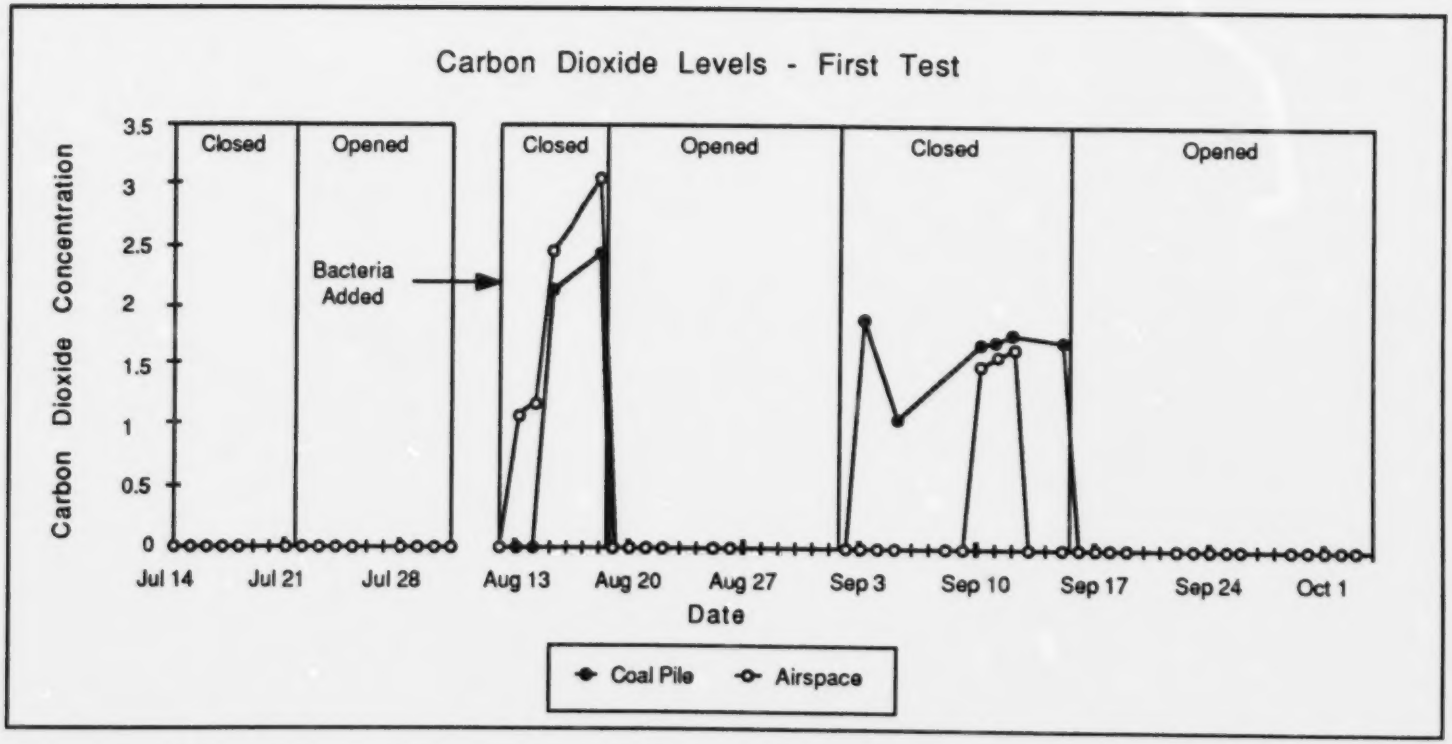

Figure 6.
Carbon Dioxide Levels During the First Test with CBDC Coal

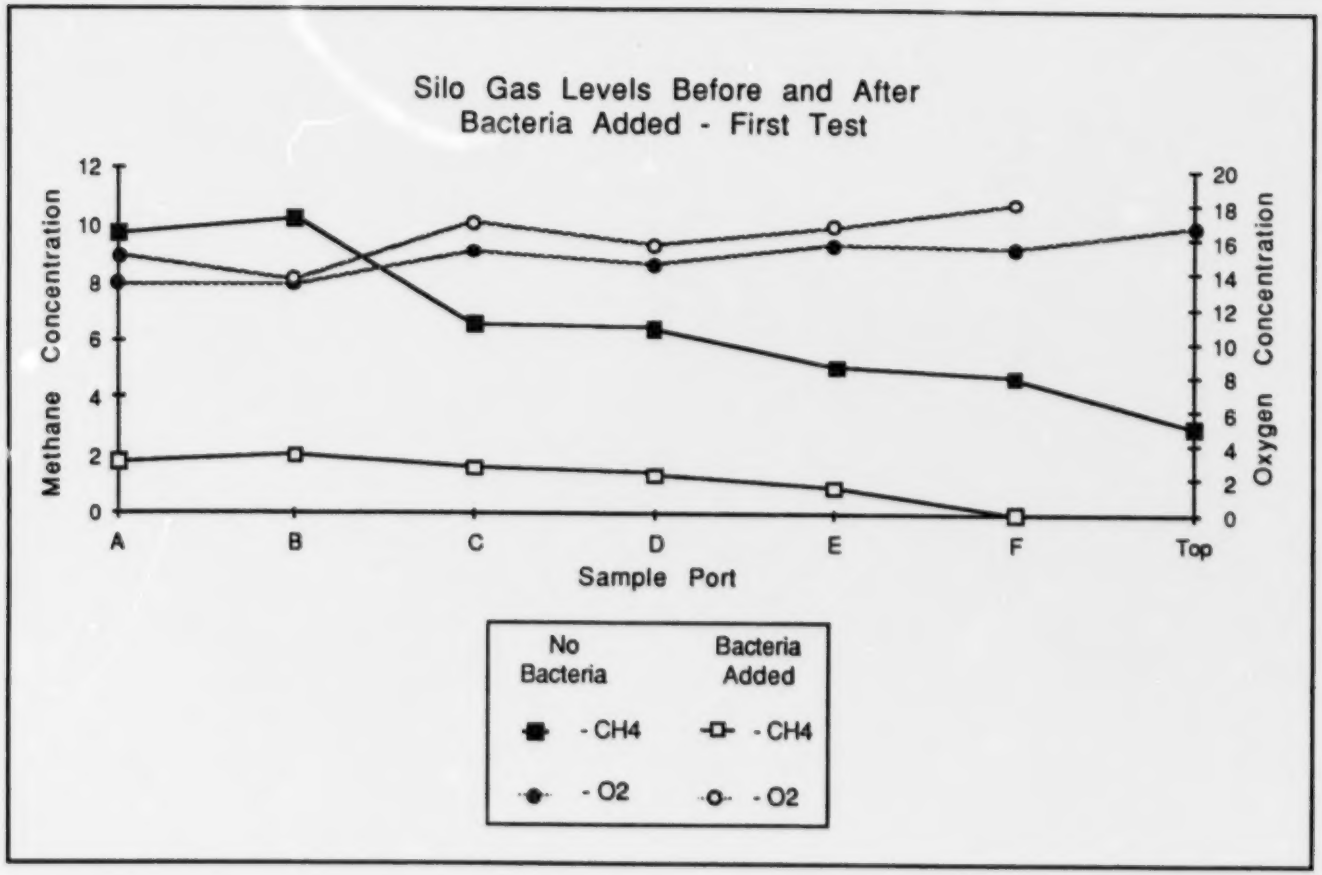

Figure 7.
Gas Level Profile During the First Test with CBDC Coal

in the air space and in the coal pile decreased to approximately steady state levels of 3 and 6% respectively. Oxygen levels throughout the silo increased rapidly.

The coal was removed from the silo, bacteria added and the coal replaced in the silo. The most dramatic change in gas composition after the addition of bacteria was the rapid depletion of oxygen in both the coal pile and the airspace. Methane accumulated but at a slower rate than before. After a period of one week the silo was opened to allow access of fresh air once more. Steady state levels of methane in the air space and the coal pile dropped to zero and 1-2% respectively. These levels remained constant for a period of about two weeks. Oxygen levels rose to 20% in the airspace and 18% in the coal pile. The oxygen concentration was fairly constant throughout the coal pile during this part of the run (Figure 7). Carbon dioxide was only detected after the addition of bacteria (Figure 6).

Another cycle of sealing and opening the silo gave identical results.

In the second set of experiments with the CBDC washplant coal 50% more coal was added to the silo. This gave a depth of coal of approximately 2.3 meters. The experimental protocol was similar to the first set of experiments with the exception that rather than opening the silo air was pumped across the top of the coal pile at a rate of 24 litres per minute. This was done to mimic the effects of the ventilation experienced on an actual coal carrying ship.

The results obtained from this run are shown in Figures 8-14. The results followed the same pattern as in the first run but were much more dramatic. For example, coal pile methane levels were below detection (<0.05%) when air was pumped through the top of the silo after bacteria were added whereas the level was 5% before the bacteria were added. The

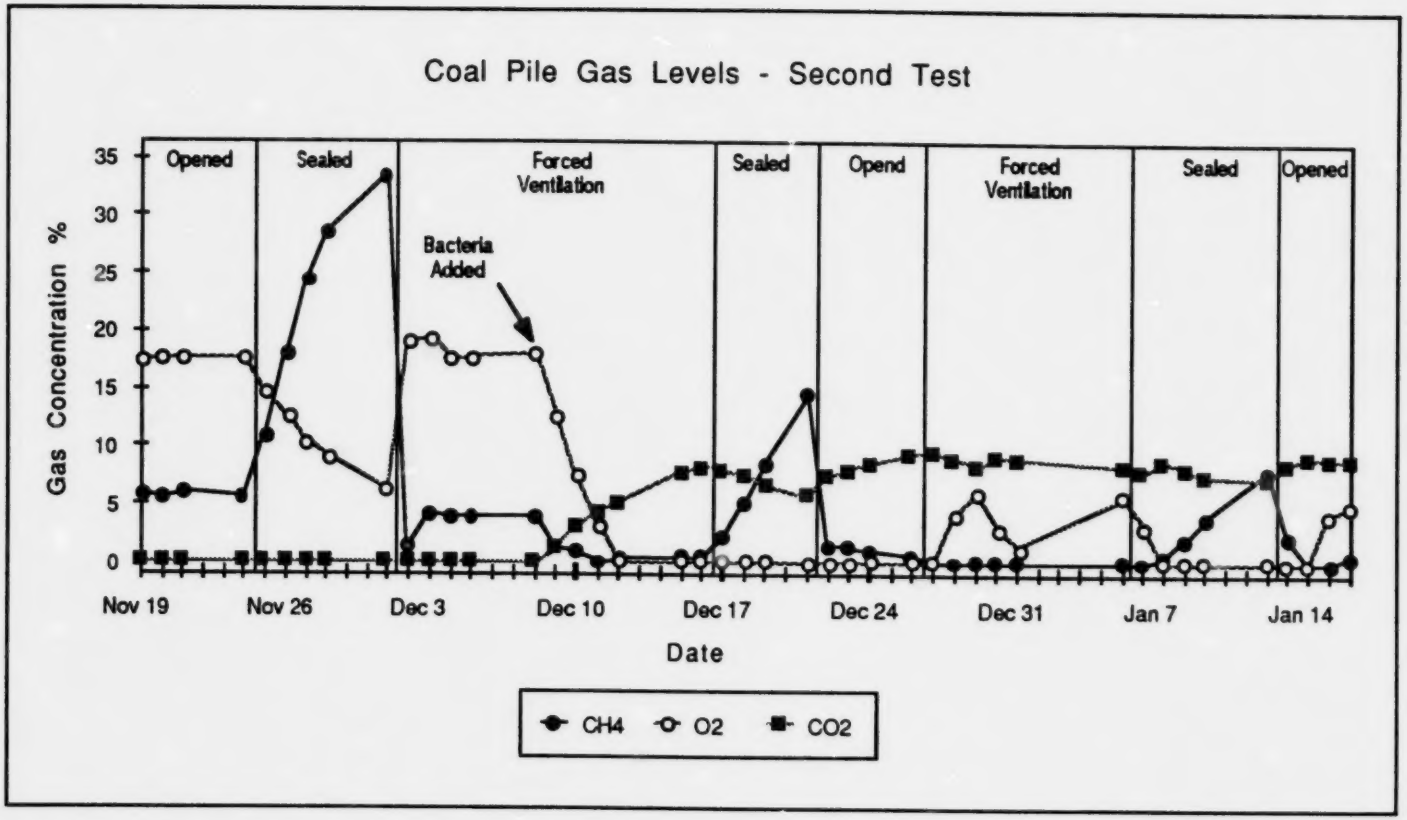

Figure 8.
Gas Levels in the Coal Pile During the Second Test with CBDC Coal

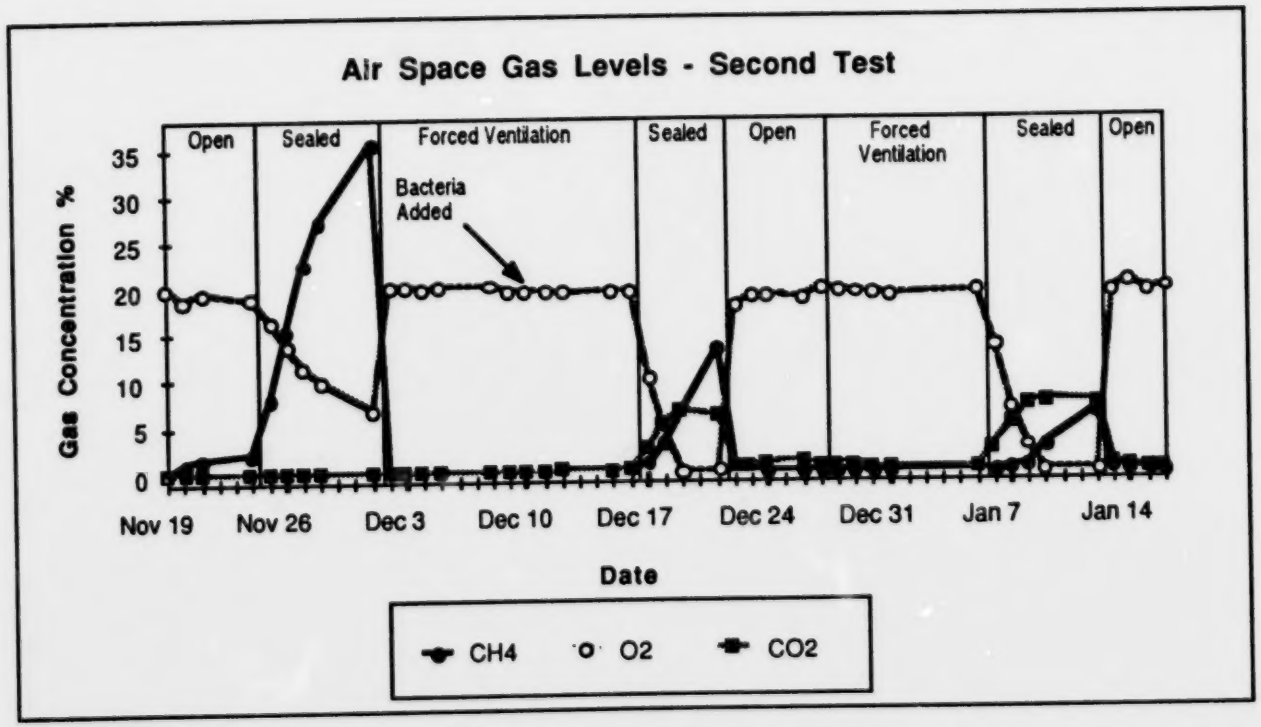

Figure 9.
Gas Levels in the AirSpace During the Second Test with CBDC Coal

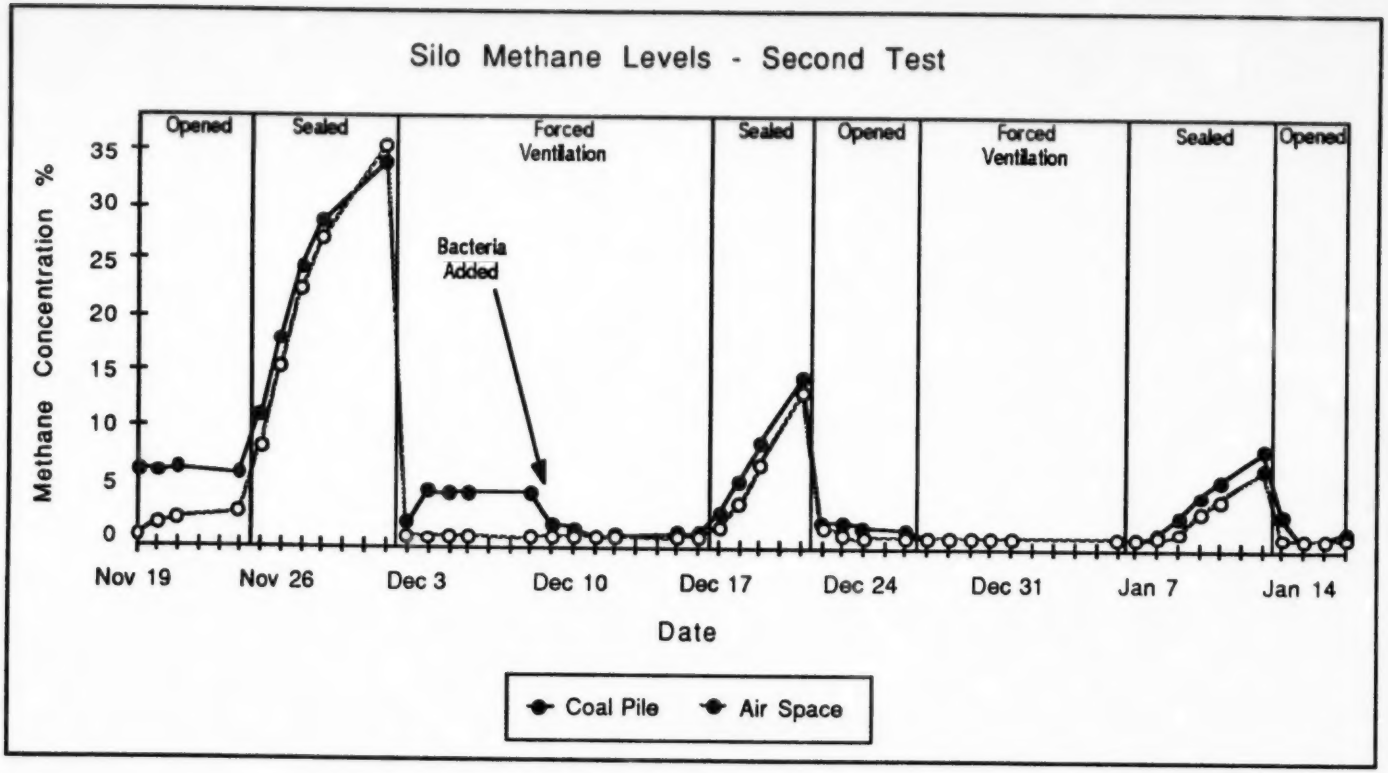

Figure 10.
Methane Levels During the Second Test with CBDC Coal

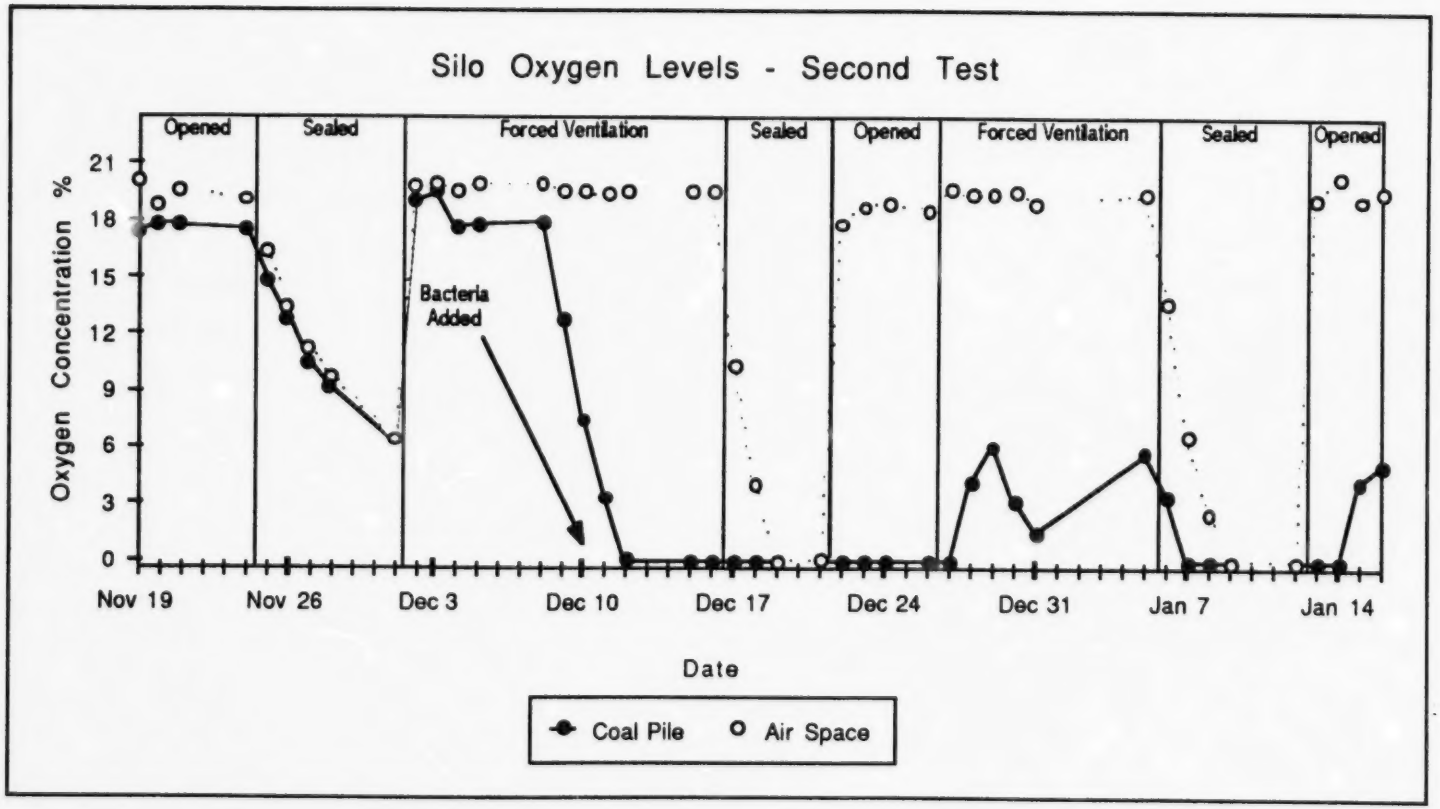

Figure 11.
Oxygen Levels During the Second Test with CBDC Coal

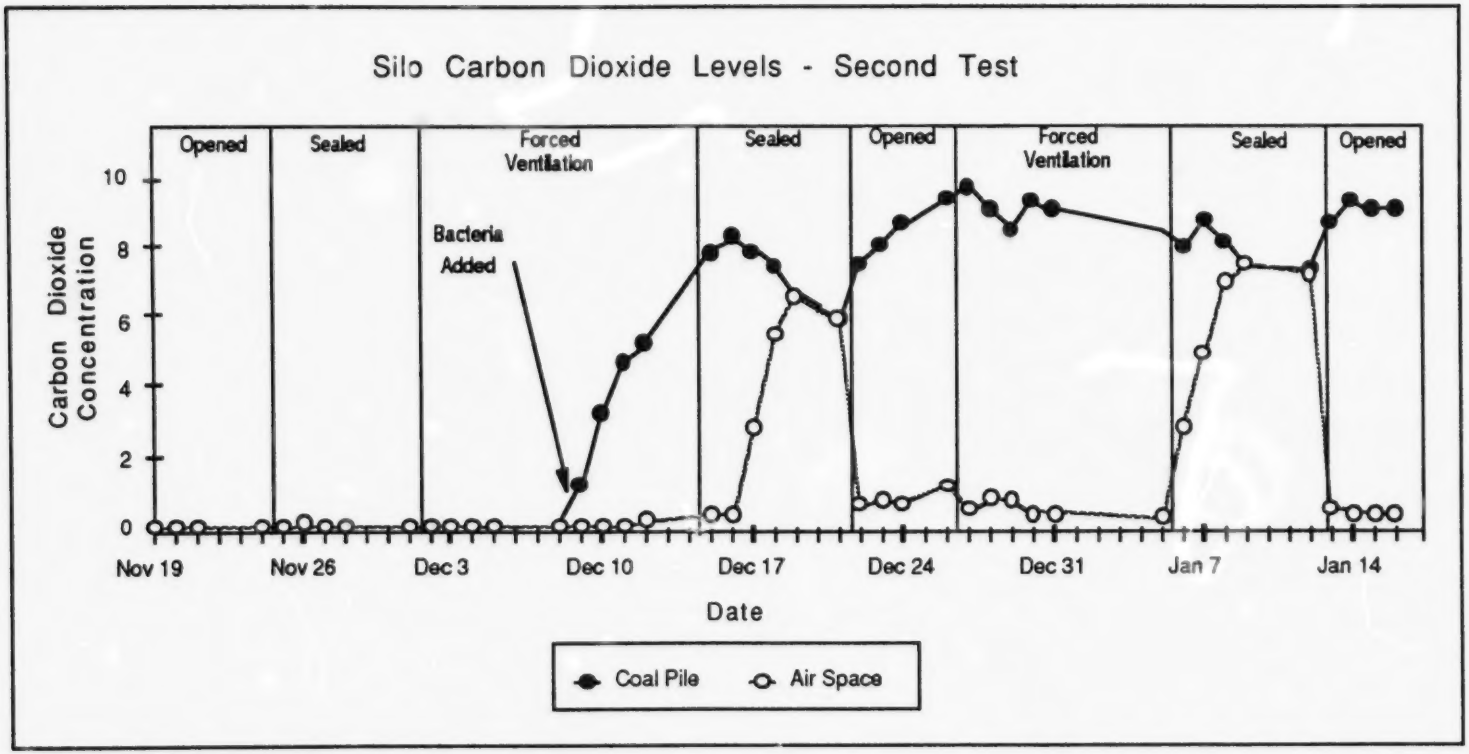

Figure 12.
Carbon Dioxide Levels During the Second Test with CBDC Coal

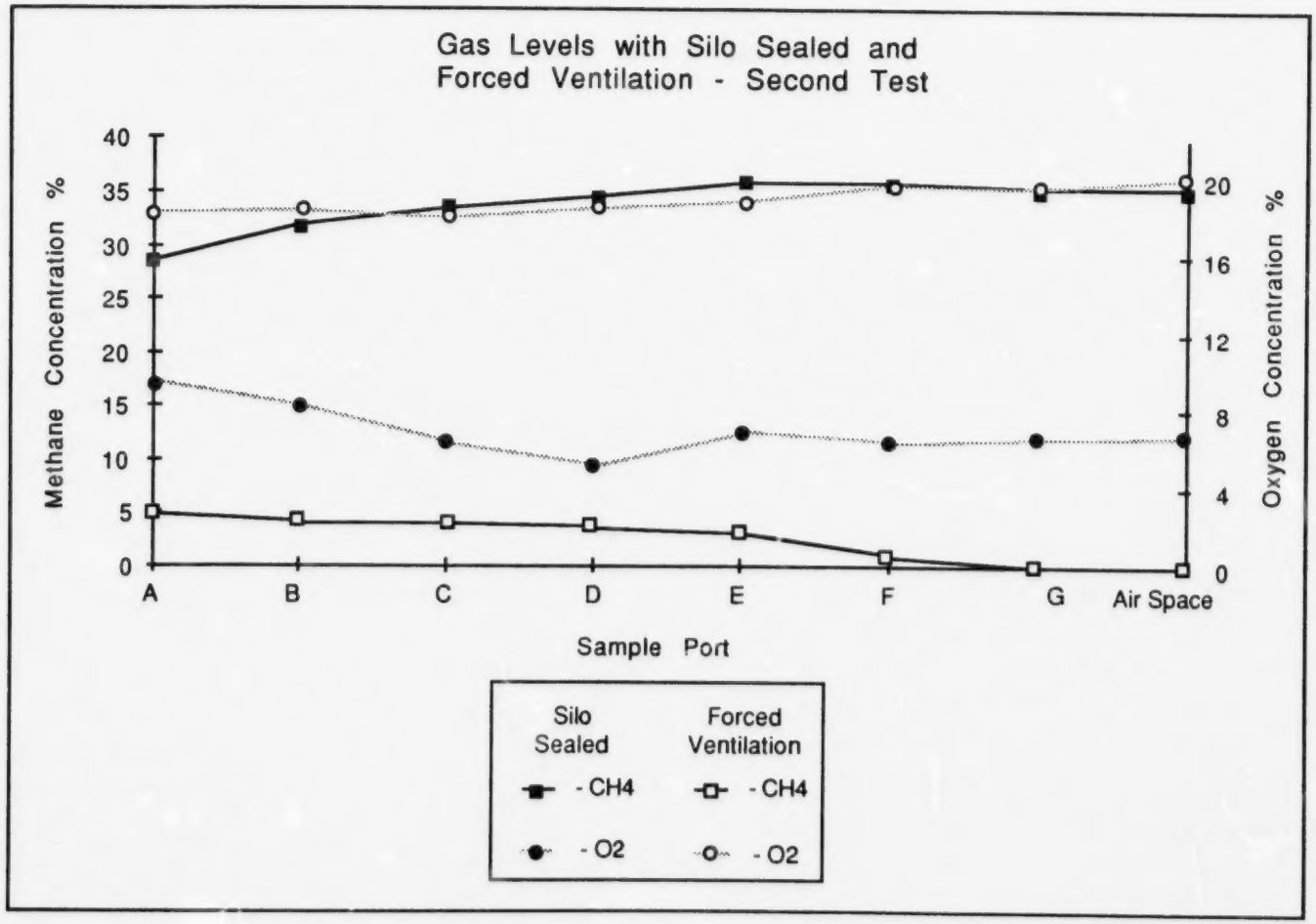

Figure 13.
Gas Level Profile During the Second Test with CBDC Coal

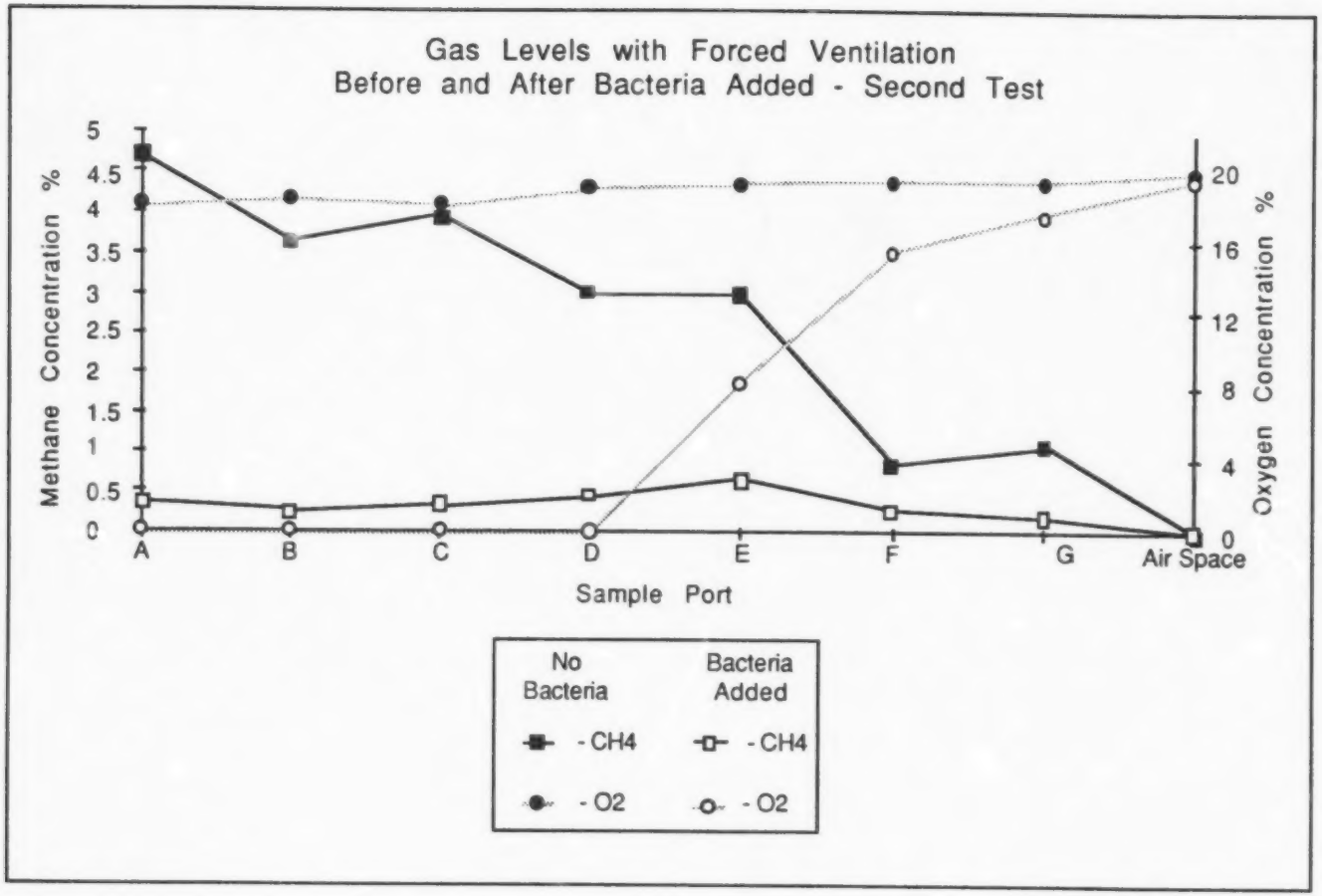

Figure 14.
Gas Level Profile During the Second Test with CBDC Coal

levels of carbon dioxide in this experiment were much higher than in the previous run. This is mostly due to the higher activity of the bacteria used. It is interesting to note that gas concentration gradients were established in this run (Figures 13 and 14). This effect is discussed in more detail in the Discussion Section.

During this run a control silo holding approximately 10 Kg of coal was monitored on a continuing basis to establish the rate of decay in the methane emission rates. The data from this experiment are shown in Figure 15. Thus the rate of methane emission from the coal had decreased by approximately 50% during the running of this experiment. This factor should be kept in mind when the results are analyzed since it has not been factored into these results.

Data from the run with Sparwood coal are shown in Figures 16, 17 and 18. The data from this experiment are not conclusive for a number of reasons. Firstly, the bacteria were added to the top of the coal within the silo rather than being mixed outside of the silo as was done with the CBDC coal. Secondly, the coal was very fine and dry and did not mix well with the bacteria thus the active methane oxidation zone was confined to a relatively narrow zone at the top of the coal pile. Thirdly, the amount of methane desorbing from this coal was very low. The experiment was further complicated by the rapid uptake of oxygen by the coal, even before bacteria were added.

The most obvious effect noted during this experiment was the rapid loss of oxygen after addition of the bacteria. Unfortunately, due to the fine nature of the coal it was almost impossible to replace the oxygen that was utilized, thus the atmosphere within the coal pile was mostly anoxic, even with pumping of air across the top of the coal pile.

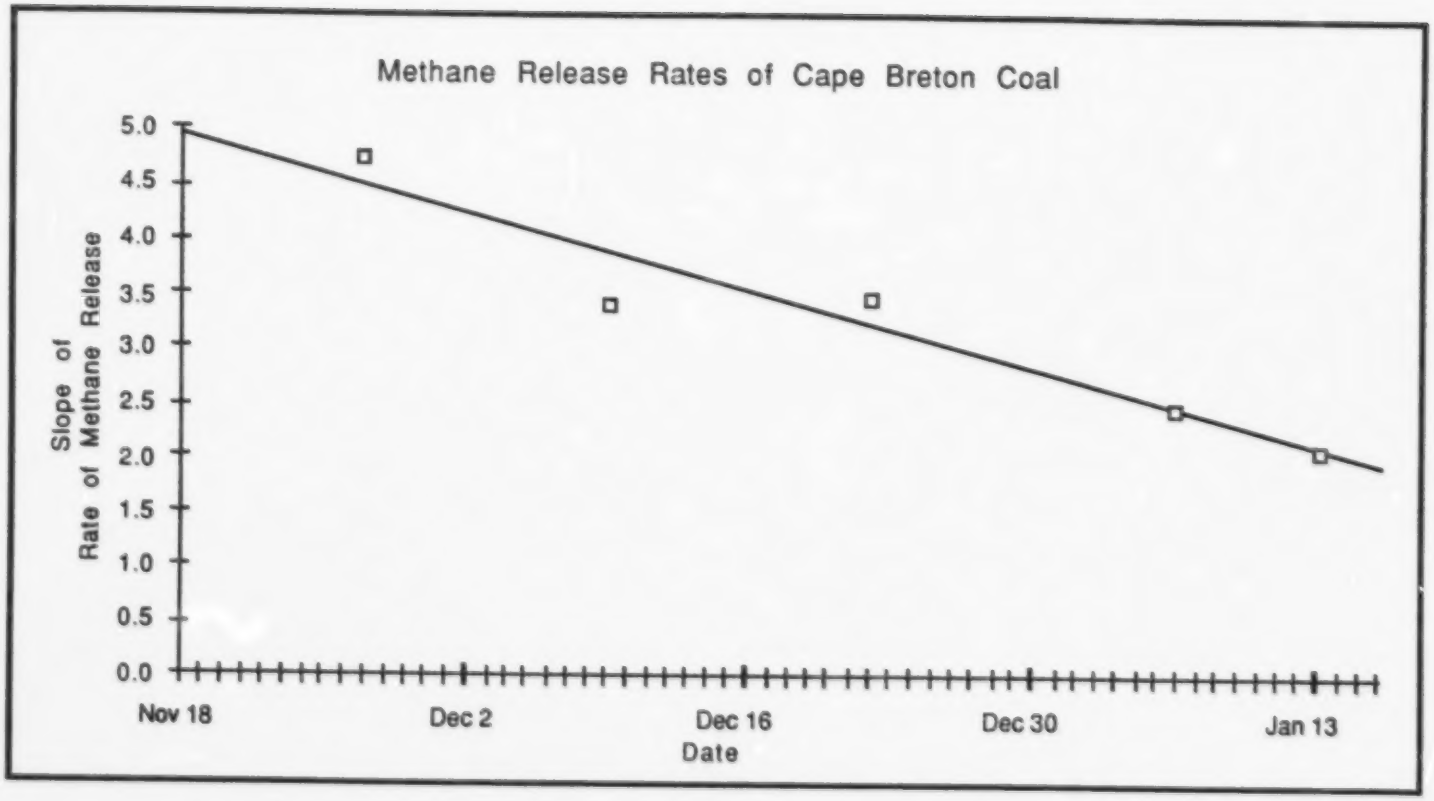

Figure 15.
Methane Emission Rates

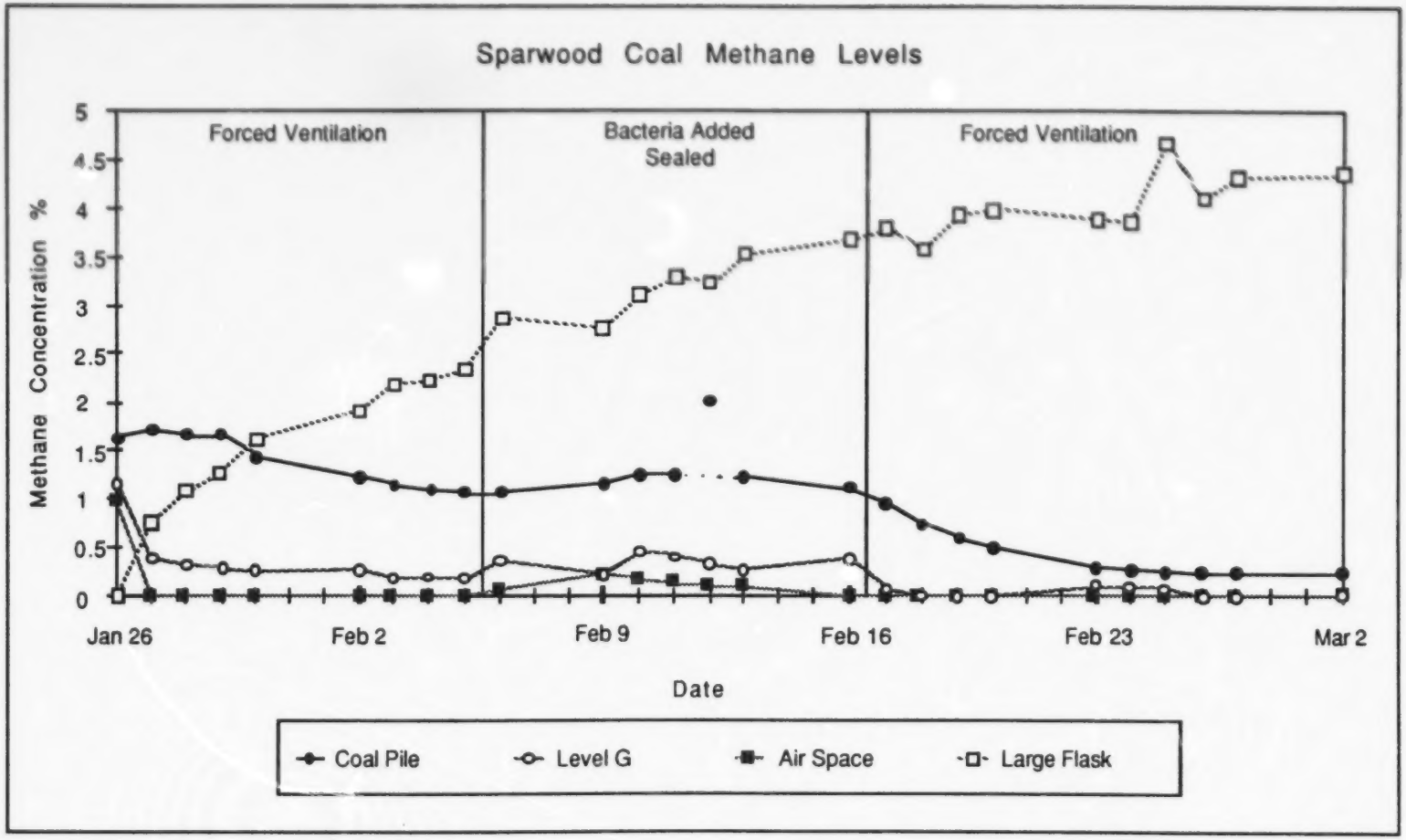

Figure 16.
Methane Levels During Tests with Sparwood Coal

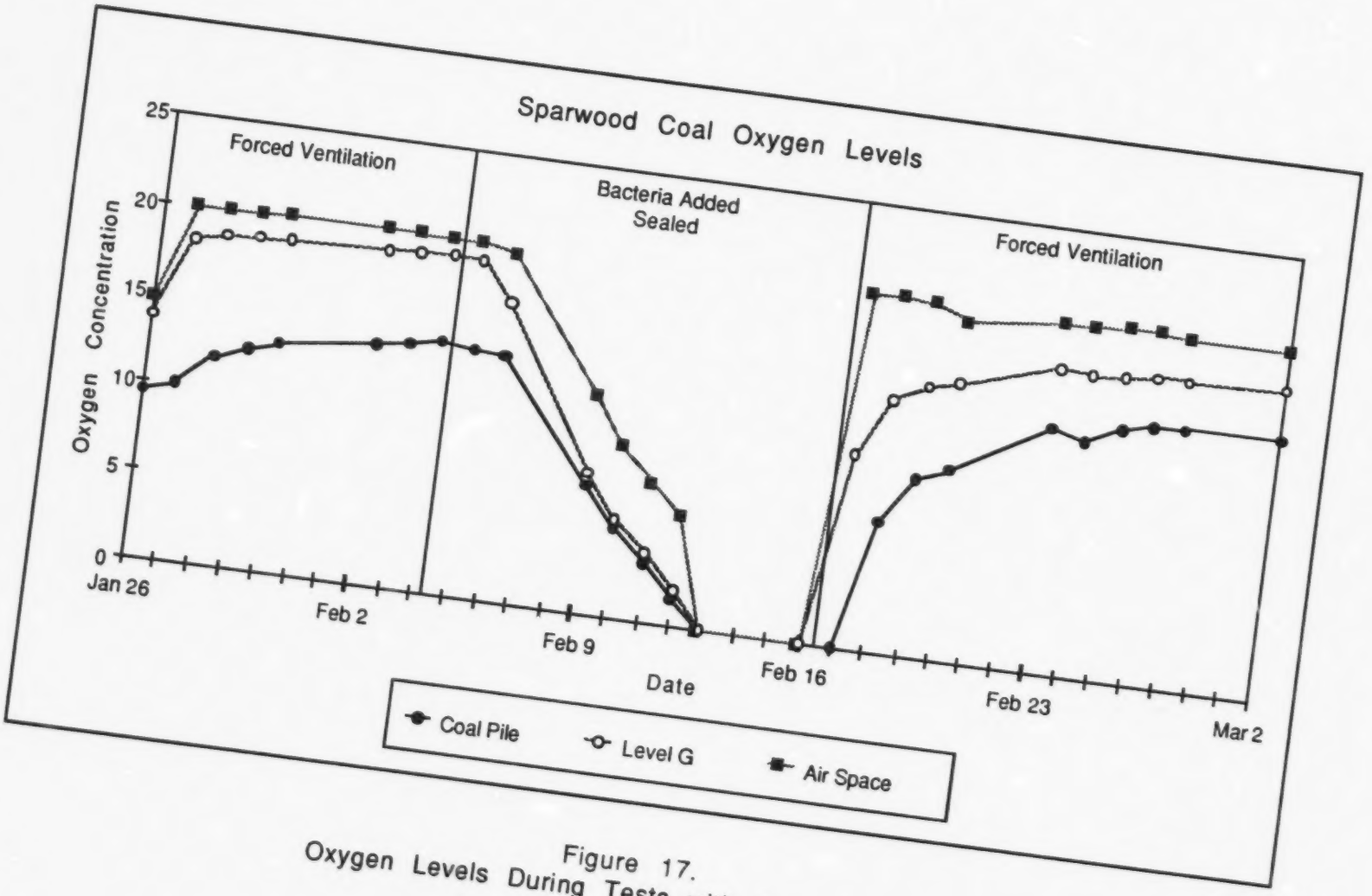

Figure 17.
Oxygen Levels During Tests with Sparwood Coal

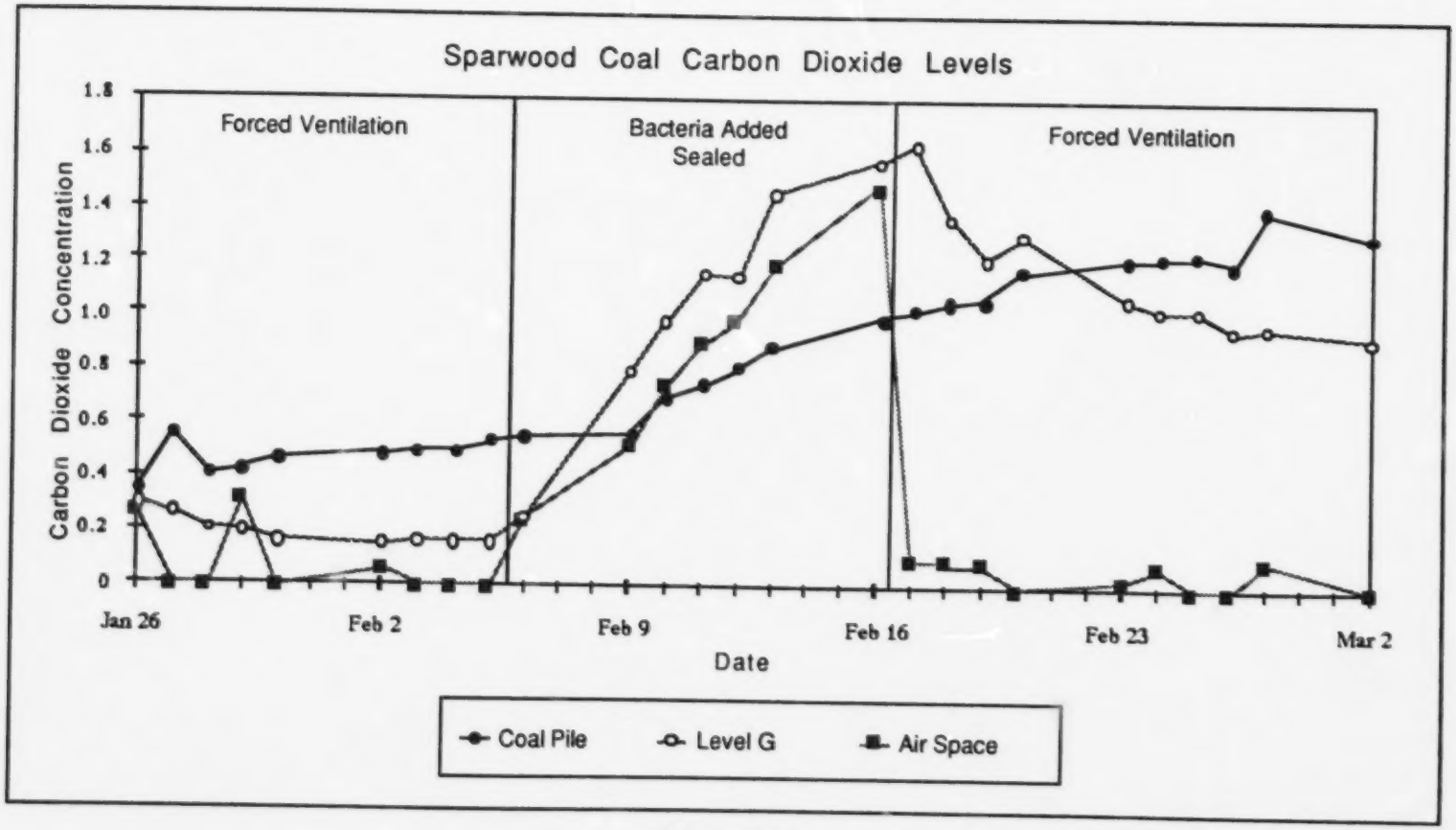

Figure 18
Carbon Dioxide Levels During Tests with Sparwood Coal

4 Differences Between "Sticky" and "Non sticky" Cells

Figure 19 shows that, contrary to what was stated in the previous report (3), there does not appear to be any major differences in either the total methane oxidation activity nor the cell biomass between "sticky" and "non sticky" cells. There were obvious differences in the morphology of the cultures since the "sticky" cells were more pigmented than the "non sticky" cells and they also grew in a much more flocculant manner. The difference found in the previous report (3) may be explained by the fact that, on a small scale experiment, the "non sticky" cells would tend to filter through the coal more readily than the "sticky" cells thus resulting in an apparent higher activity for the "sticky" cells. On the much larger scale used in this set of experiments this factor was not important. The "sticky" cells would still be the culture of choice for use with immobilized cells (see Section 8).

5 Growth on Media Containing Varying Levels of Nitrogen

Figure 20 shows the results of growing the methane oxidizing bacteria on varying levels of nitrogen. Little difference in the levels of methane oxidation rates was found between the cultures grown on the different levels of nitrogen. However, the amount of pigmentation was higher at lower nitrogen levels. In addition, the cells grown at the lowest nitrogen levels appeared to be very similar to the "sticky" cells. This "sticky" nature seemed to be more stable on the low nitrogen media and was not lost on continued transfer.

6 Effects of Concentrating the Methane Oxidizing Bacteria

Figure 21 shows that concentration of the methane oxidizing bacteria has no deleterious effect on the methane oxidizing activity. In fact, there is a slight enhancement of activity

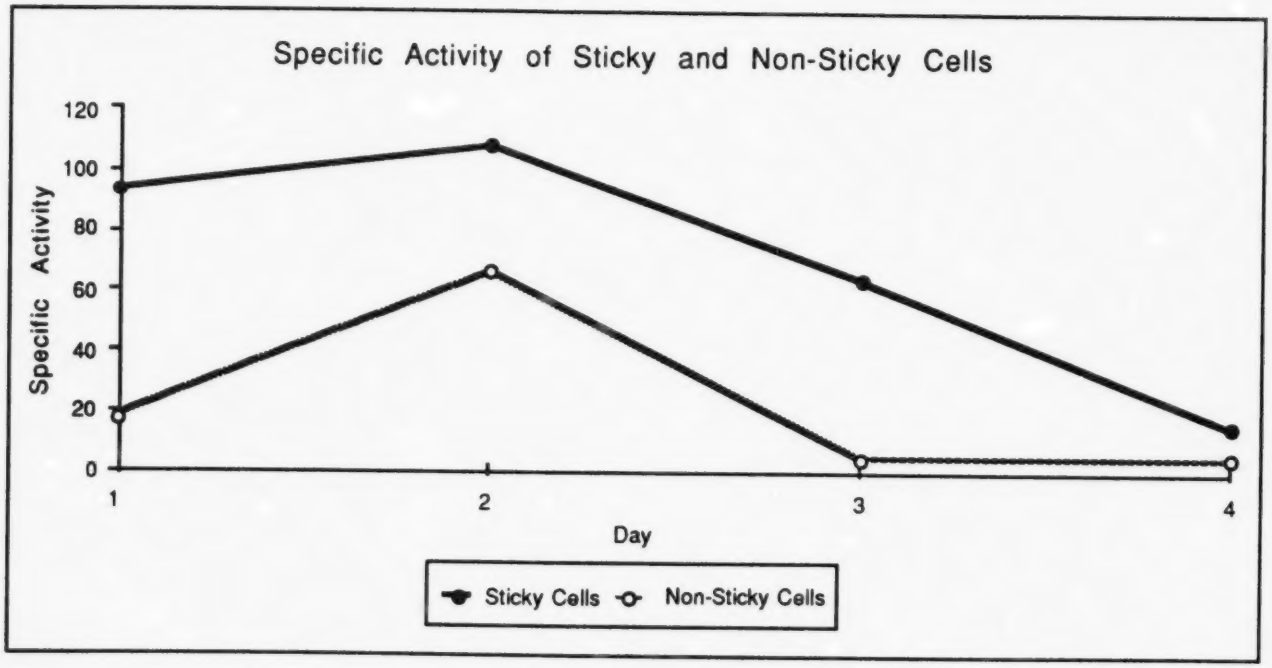

Figure 19.
Comparison of "Sticky" and "Non-sticky" Cells

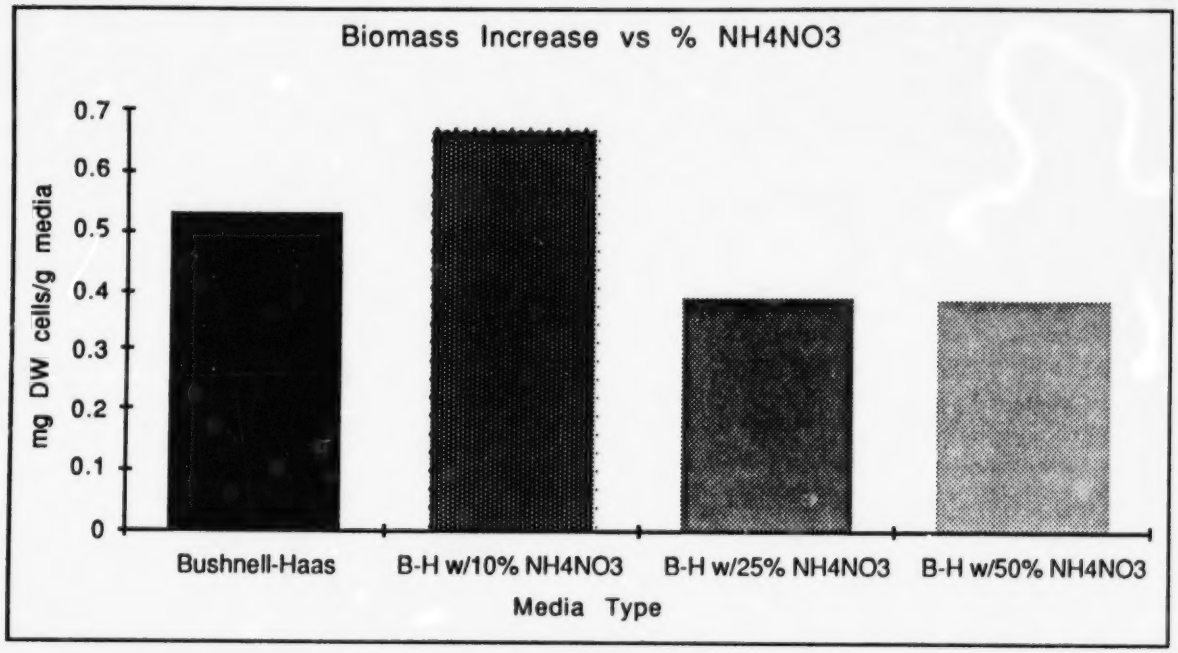

Figure 20.
Effects of Reduced Nitrogen Levels in Medium

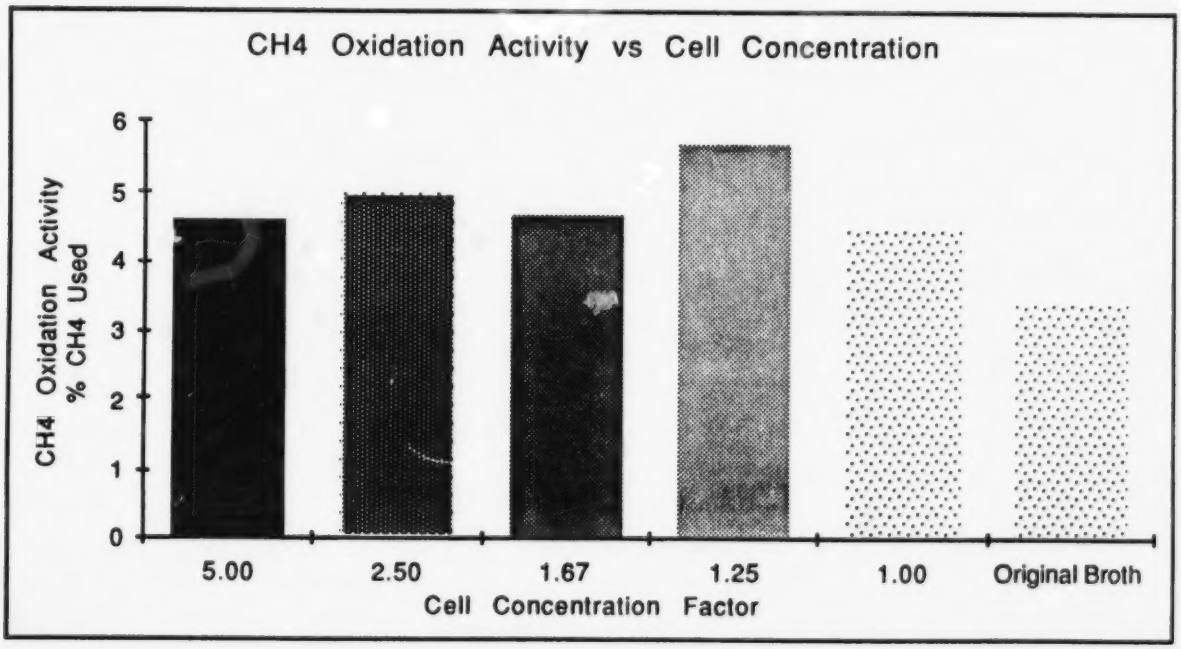

Figure 21.
Effects of Concentrating the Cells

after resuspension of the cells into fresh medium. Cells reconstituted into 40% of their original volume appeared to show the best activity after resuspension.

7 Stability of the Methane Oxidizing Bacteria

Figure 22 shows that the methane oxidizing activity in crude fermentation broths is stable for considerable periods of time. It should be noted that in the majority of cases examined, the activity was quite stable, however, in a few circumstances the activity was rapidly lost. It is not known with certainty why these particular samples lost activity but it could be due to bacterial contamination or to some chemical inhibitor which was present in the sample container. Inhibitors which could cause problems would include various surfactants and detergents.

8 Immobilized Cells

The results of experiments using immobilized cells are shown in Figure 23. The results with the 500 ml model were good, however, the results with the 20 litre model were not satisfactory.

9 Economic Assessment

The data presented in this report support the economic assessment made in the last report (3). Table 2 shows the breakdown of costs for plants with capacities to treat 1000 and 5000 tonnes of coal per day. The costs reported in Table 2 are based on the costs of the 2000 litre fermentor Gemini has already had constructed. The costs are also based on the use of industrial grade chemicals and a price quoted by a leading chemical supply house.

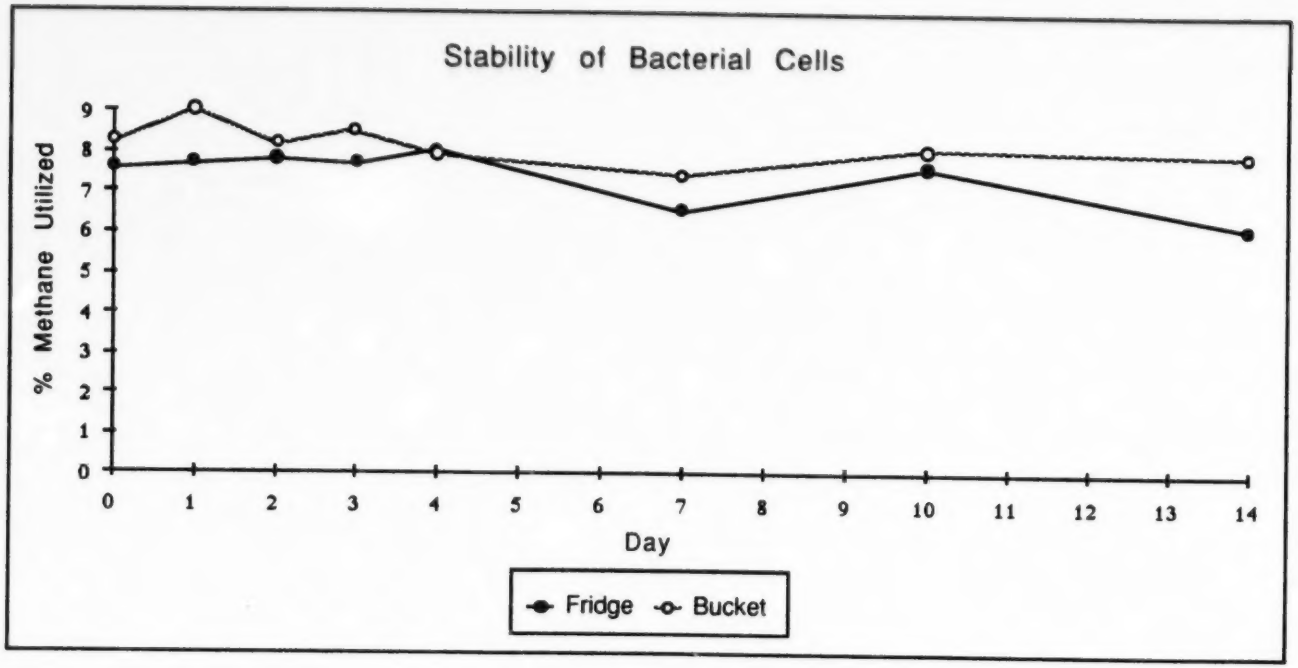

Figure 22.
Stability of the Bacterial Oxidation Activity

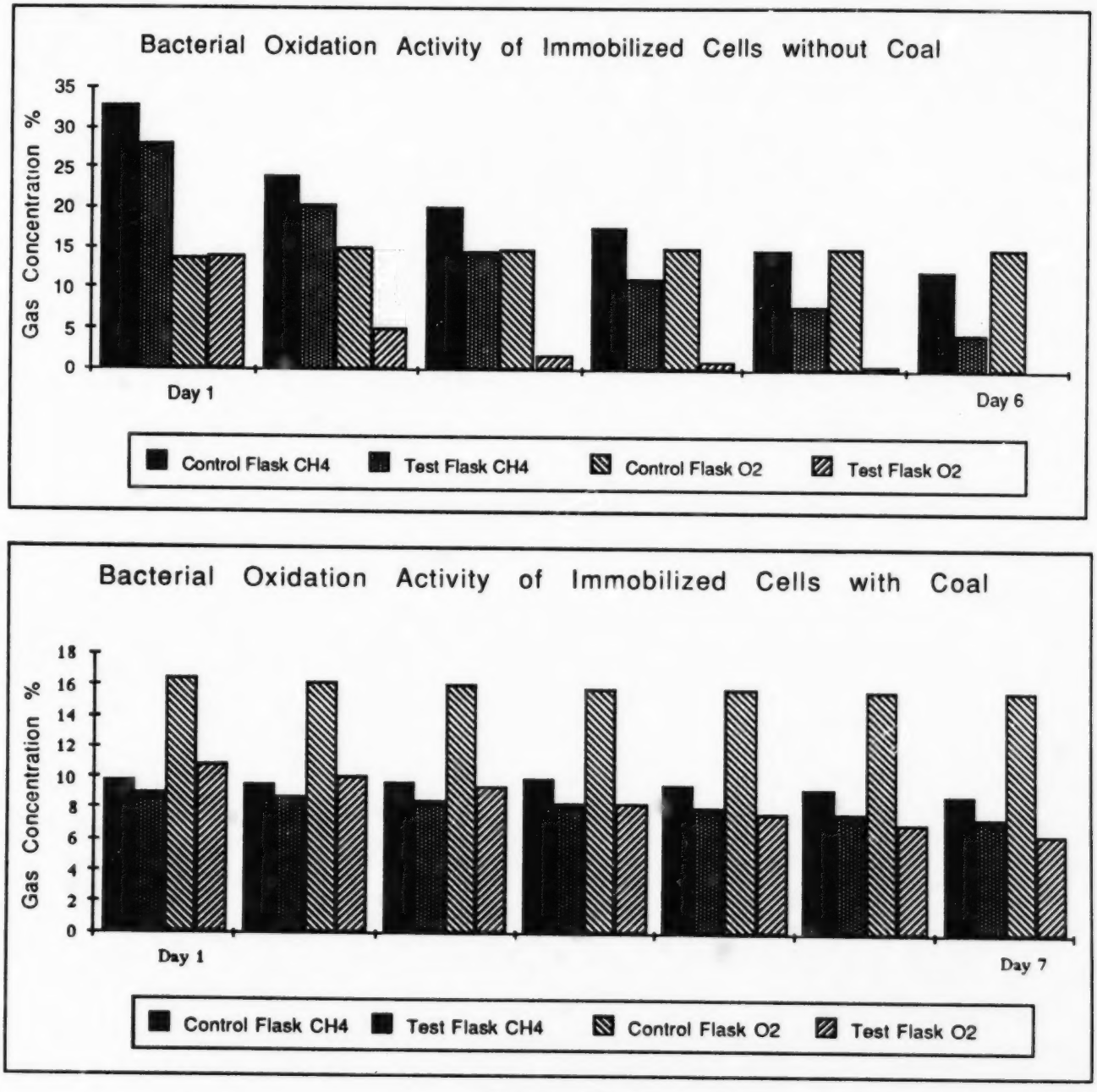

Figure 23.
Bacterial Oxidation Activity of Immobilized Cells

TABLE 2

ECONOMIC ASSESSMENT

	1000 tonne per day	5000 tonne per day
Depreciation	$100000	$500000
Labour	125000	150000
Raw materials	60000	200000
Operating costs	30000	50000
Total annual cost	$315000	$900000
Cost per tonne*	$0.86-1.75	$0.49-0.98

* Lower cost for plant operating 100% of the time. Higher cost for plant operating 50% of the time

DISCUSSION

The results of the previous study (3) showed that methane oxidizing organisms cultured in the laboratory could oxidize methane when in contact with low sulphur coal. The process was carried out in a model silo which contained 10 Kg of coal. The organisms were partially characterized and the conditions required to produce them on a laboratory scale were examined.

The study was continued so that more data could be obtained on the following parameters:

- o design of a bioreactor to produce the bacteria on a larger scale
- o testing of the process on a larger scale -2-3 tonnes of coal per test
- o scale up of the immobilized cell experiments.

The results from this phase of the study continue to be most positive and encouraging. These results are discussed below.

One of the key components in this process is the bioreactor to produce the bacteria. The bioreactor must be efficient at producing active cells and it must do so in a cost effective manner. The bioreactors constructed during this phase of the project fulfill both of these criteria. The kinetics obtained in these bioreactors were as good as those obtained previously in the small stirred fermentors thus showing that bioreactors based on the air-lift principle can be used to produce methane oxidizing bacteria. The main advantages of air-lift reactors as opposed to stirred tank reactors are listed below:

- o airlift reactors are less expensive to construct
- o they are less expensive to operate
- o they are less likely to breakdown since they have no

moving parts and no seals to cause leaks.

Scale up of an air-lift bioreactor will present fewer problems than the scale up of a stirred reactor since there are fewer parameters involved. Thus it should be relatively simple to scale up the bioreactor to produce sufficient cells for the expected next phase of the project.

The results of the three experiments with the model silo will be discussed separately since there were enough differences between runs to justify their separate discussion.

The first run with the CBDC washplant coal used approximately 2 tonnes of coal. During the first week of this experiment the silo was sealed and there were no bacteria added to the coal. There was a rapid rise in methane during this period. The oxygen levels showed a decrease but this was mainly due to the addition of the methane which resulted in an apparent lowering of oxygen concentration. After a period of one week the silo top was removed to allow access of air to the coal. This resulted in a lowering of the steady state methane concentration within the coal pile to 6-7%. The methane in the air space during this period was in the range 3-5%.

There was an immediate drop in oxygen concentrations after bacteria were added (with the silo closed). The methane levels rose slowly until all the oxygen had been consumed at which point it rose as rapidly as before. After opening the silo the methane concentration in the coal pile reached a steady state level of 2%, which was considerably less than when there were no added bacteria. The methane in the air space was undetectable during this time. There was no forced ventilation during this experiment. If there had been, the level of methane would have been even lower.

Further evidence to indicate that the lower methane concentrations were due to bacterial oxidation was the fact that carbon dioxide was only detectable after the addition of the bacteria and not before.

It should be noted that during this experiment the oxygen levels in the silo did not show any gradation from top to bottom of the coal pile. This shows that the rate of methane oxidation does not appear to be limited by the diffusion of oxygen to the lower points in this particular silo.

The results obtained from the second run with CBDC coal were even more positive than those from the first run. The levels of methane in the coal pile during forced ventilation of the silo dropped from 5% with no added bacteria to less than 1% after addition of bacteria. During a second cycle of testing the level in the coal pile dropped to less than detectable levels. Further evidence that bacterial oxidation was responsible for the dramatic decrease in methane comes from an examination of the carbon dioxide levels. Before addition of bacteria there was no carbon dioxide found in the silo but after addition levels increased quickly to 8% in the coal pile and 1% in the air space (with forced ventilation). The activity of the methane oxidizing bacteria was so high during this experiment that carbon dioxide was detected in the air space above the coal even when this space was ventilated with a flow of air.

When gas concentrations are examined at different levels in the silo it can be seen that gradients were established during this experiment. There are two possible explanations for this ocurring during this experiment but not in the first.

Firstly, the depth the coal bed was 50% greater in this run than in the first run thus causing diffusion to have more of a rate limiting effect. Secondly, the bacteria used in this

run were more active than in the first run which would again result in the reaction being diffusion limiting since the rate of oxygen utilization would be greater than the rate at which oxygen would diffuse from the top of the silo. These two factors taken together would explain why an oxygen gradient was established during this run but not the first. The rate of oxygen diffusion to the bottom of the silo must have been very close to the rate of microbial oxygen utilization since the level of methane would have risen if there had been insufficient oxygen for microbial oxidation.

This experiment provides another relevant finding. One problem which could limit the usefulness of this microbial method for methane control is the availablity of oxygen to the bacteria. If the data from Figure 13 are examined it can be seen that when air is passed across the top of the coal oxygen can diffuse to an appreciable depth in the coal pile, thus increasing the depth of the methane oxidizing zone. From the data obtained during this run it can be seen that there is methane oxidation occuring to a depth of at least six feet in the coal pile provided that the coal has not been well packed.

The test with the Sparwood coal did not give definitive results. This coal was very finely ground (100 mesh) and could not be wetted evenly with the bacterial cells. The bacteria were added to the top of the coal pile and they did not penetrate very deeply into the coal. If further tests are to be carried out with a finely ground coal the effects of wetting agents should be tested. This could allow for more even wetting of the coal by the bacterial cells. Another problem with this coal is that the fine nature of the coal impeded the diffusion of gases, particularily oxygen. Results from previous tests (2,3) show that there is nothing inhibitory about this coal thus it is only the physical nature of the coal which produced the poor results observed during the current tests.

In the previous report (3) there had been an indication that the "sticky" cells were more reactive when applied to coal than the "non-sticky" cultures. Several experiments were performed during this phase of the project to see if, in fact, this was so. The results described in this report show that there is very little difference between these two cell cultures when an examination of their biomass and methane oxidizing activities is performed. When larger volumes of the "non-sticky" culture (10%;v/w) is added to much larger quantities of coal there is no difference in the expected activity. The differences noted in the last report probably reflect that on small samples of coal a significant portion of the culture drains to the bottom of the flask and thus the activity in this fraction is lost all together or at least is greatly reduced. At low culture volume to coal ratios (e.g. 2.7%) there would be no difference between "sticky" and "non-sticky" cells. However, if the cells are to be immobilized onto an inert support, "sticky" cells would be preferred since they will adhere to the support material.

One of the methods tried to encourage the growth of "sticky" cells was to grow them on a medium containing low levels of nitrogen. On a medium containing 10% of the regular nitrogen concentration the culture grew as "sticky" cells. The cells produced in this manner were stable on repeated transfer, whereas, "sticky" cells obtained from agar plates and transferred on medium containg regular concentrations of nitrogen became "non-sticky".

The amount of additional water added to the coal during this process is of concern. The amount of culture broth added in this phase of the project was 2.7 wt%. An experiment was conducted to see if this could be reduced further. Cells were concentrated to 20, 40, 60 and 80% of their initial volume. This had very little effect on the methane oxidation

activity of the organisms when these concentrated samples were added to coal. In fact, the resuspension of the cells in fresh medium had a slightly stimulatory effect, probably due to the removal of small, diffusible and inhibitory metabolites produced by the bacteria. A second but less likely explanation is that some essential nutrient in the medium had been used up and was replaced. However, due to the large excess of nutrients in the Bushnell Haas medium this is considered unlikely.

The bacteria could retain their activity for extended periods of time. It did not appear to make any difference whether they were stored at room temperature or 4°C. The stability experiment is being continued for longer periods of time.

The tests with immobilized cells worked well on a small scale (500 ml) but problems were encountered when attempts were made to scale up the tests to 20 litres. Part of the problem was that with the larger tests it was immpossible to immobilize the same ratio of cells as with the small tests since the bacteria were not "sticky" enough. Further work is required to find the best solid support fot the bacteria and for producing the "sticky" cells in large quantities.

CONCLUSIONS

1 Two 30 litre airlift bioreactors were successfully designed and operated so that 50 litres of culture broth could be prepared in one batch. The kinetics of production were studied and compared to the results obtained previously in the small fermentor. The kinetics were improved in the bioreactors since good activity could be obtained in a period of 3-4 days as opposed to the 4-5 days reported previously.

2 Two successful tests were carried out in the 3 tonne silo using the CBDC coal. With the silo sealed and bacteria added there was complete utilization of oxygen within a very short period of time. With forced ventilation across the top of the coal the level of methane within the coal pile was reduced by a factor of 4-5 after addition of the bacteria. Analysis of oxygen levels in the silo showed that with the CBDC washplant coal appreciable amounts of oxygen could diffuse to the bottom of the silo. The results with the Sparwood coal were not conclusive. This was partly due to the finely ground nature of this particular coal which did not allow even wetting of the coal and to the fact that diffusion of oxygen was impaired in this coal.

3 The bacteria were quite stable, with no loss in methane oxidation activity noticed after a week of storage at either room temperature or at 4°C. The bacteria could be concentrated to 20-40% of their original volume with no loss in activity. The economics of concentrating the bacteria have not been evaluated at present.

4 There did not appear to be any major differences in the activity between "sticky" and "non-sticky" cells. The differences noted during the previous study most probably reflect the fact that the bacterial cell suspension was

applied to the coal in large excess thus a lot of the "non-sticky" cells flowed to the bottom of the container and were in-active during the tests. When the amount added to the coal was reduced, no differences were noticed. "Sticky" cells would be preferred for use in a bioscrubber type of operation.

RECOMMENDATIONS FOR FURTHER RESEARCH

Based on the results described in this report, the following recommendations are made for further research.

1 Further scale up of the bioreactors should be carried out. Gemini has a prototype airlift bioreactor with a capacity of 1800 litres. It is recommended that conditions for producing the methane oxidizing bacteria in this bioreactor be established.

2 The work with the model silo should be scaled up. It is recommended that tests be carried out in a silo or model ship hold capable of holding 40-100 tonnes of coal. It would be preferrable if this phase of the project could be carried out on the premises of CBDC.

3 More work should be done with the 3 tonne silo to determine the maximum penetration of oxygen into the coal pile.

4 More work should be conducted on producing "sticky" cells. It is recommended that efforts be made to grow these cells in the 30 litre airlift bioreactors. These cells could be used to scale up experiments on the use of bioscrubbers.

REFERENCES

1. "Biological Control of Methane in Coal Mines- A Feasibility Study" CANMET Report: Prepared by Gemini Biochemical Research Ltd., 1983.

2. "Biological Control of Methane in Caol Mines, Coal Storage Silos and Ship Holds -Phase II" CANMET Report: Prepared by Gemini Biochemical Research Ltd., 1984.

3. "Biologiacl Control of Methane in Coal Mines, Coal Storage Silos and Ship Holds -Phase III" CANMET Report: Prepared by Gemini Biochemical Research Ltd., 1986

APPENDIX A

Analytical Methods

Gases were analyzed using a Perkin Elmer Sigma 3B gas chromatograph using the following conditions:

Column	Supelco carbosieve SII 10 x 1/8' s.s.
Oven temperature	75°C for oxygen, 225°C for methane and carbon dioxide
Injector temperature	250°C
Detector temperature	250°C
Carrier gas	helium at 30 ml/minute
Detector type	hot wire
Sample loop	0.1 ml

pH measurement was made using a combination electrode and a Fisher Accumet pH meter (model 425 digital).